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(54) Title: MEANS FOR THE MODULATION OF PROCESSES MEDIATED BY RETINOID RECEPTORS AND COMPOUNDS USEFUL THEREFOR			
(57) Abstract			
<p>In accordance with the present invention, there are provided methods to modulate processes mediated by retinoid receptors, employing high affinity, high specificity ligands for such receptors. In one aspect of the present invention, there are provided ligands which are more selective for the retinoid X receptor than is retinoic acid (i.e., rexoids). In another aspect of the present invention, alternative ligands (other than retinoic acid) have been discovered which are capable of inducing retinoic acid receptor mediated processes. In yet another aspect, methods have been developed for the preparation of such retinoid receptor ligands from readily available compounds.</p>			

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MEANS FOR THE MODULATION OF PROCESSES MEDIATED BY
RETINOID RECEPTORS AND COMPOUNDS USEFUL THEREFOR

FIELD OF THE INVENTION

The present invention relates to intracellular receptors, and ligands therefor. In a particular aspect, - 5 the present invention, relates to methods for modulating processes mediated by retinoid receptors.

BACKGROUND OF THE INVENTION

10 A central problem in eukaryotic molecular biology continues to be the elucidation of molecules and mechanisms that mediate specific gene regulation in response to exogenous inducers such as hormones or growth factors. As part of the scientific attack on this problem, a great deal 15 of work has been done in efforts to identify exogenous inducers which are capable of mediating specific gene regulation.

20 Although much remains to be learned about the specifics of gene regulation, it is known that exogenous inducers modulate gene transcription by acting in concert with intracellular components, including intracellular receptors and discrete DNA sequences known as hormone response elements (HREs).

25 As additional members of the steroid/thyroid superfamily of receptors are identified, the search for exogenous inducers for such newly discovered receptors (i.e., naturally occurring (or synthetic) inducers) has 30 become an important part of the effort to learn about the specifics of gene regulation.

The retinoid members of the steroid/thyroid superfamily of receptors, for example, are responsive to 35 compounds referred to as retinoids, which include retinoic

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acid, retinol (vitamin A), and a series of natural and synthetic derivatives which have been found to exert profound effects on development and differentiation in a wide variety of systems.

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The identification of compounds which interact with retinoid receptors, and thereby affect transcription of genes which are responsive to retinoic acid (or other metabolites of vitamin A), would be of significant value, 10 e.g., for therapeutic applications.

Recently, a retinoic acid dependent transcription factor, referred to as RAR-alpha (retinoic acid receptor-alpha), has been identified. Subsequently, two additional 15 RAR-related genes have been isolated; thus there are now at least three different RAR subtypes (alpha, beta and gamma) known to exist in mice and humans. These retinoic acid receptors (RARs) share homology with the superfamily of steroid hormone and thyroid hormone receptors and have been 20 shown to regulate specific gene expression by a similar ligand-dependent mechanism [Umesono et al., *Nature* 336: 262 (1988)]. These RAR subtypes are expressed in distinct patterns throughout development and in the mature organism.

25 More recently, additional novel members of the steroid/thyroid superfamily of receptors have been identified, such as, for example, retinoid X receptor-alpha [RXR- α ; see Mangelsdorf et al., in *Nature* 345: 224-229 (1990)], retinoid X receptor-beta [RXR- β ; see Hamada et 30 al., *Proc. Natl. Acad. Sci. USA* 86: 8289-8293 (1989)], and retinoid X receptor-gamma [RXR- γ ; see Mangelsdorf et al., *Genes and Development* 6:329-344 (1992)]. While these novel receptors are responsive to retinoic acid, the primary exogenous inducer(s) for these receptors have not been 35 identified.

Although both RAR and RXR respond to retinoic acid *in vivo*, the receptors differ in several important aspects. First, RAR and RXR are significantly divergent in primary structure (e.g., the ligand binding domains of RAR α and RXR α have only 27% amino acid identity). These structural differences are reflected in different relative degrees of responsiveness of RAR and RXR to various vitamin A metabolites and synthetic retinoids. In addition, distinctly different patterns of tissue distribution are seen for RAR and RXR. In contrast to the RARs, which are not expressed at high levels in the visceral tissues, RXR α mRNA has been shown to be most abundant in the liver, kidney, lung, muscle and intestine. Finally, response elements have recently been identified in the cellular retinol binding protein type II (CRBPII) and apolipoprotein AI genes which confer responsiveness to RXR, but not RAR. Indeed, RAR has also been recently shown to repress RXR-mediated activation through the CRBPII RXR response element. These data, in conjunction with the observation that both RAR and RXR can activate through the RAR response element of the RAR β promoter, indicate that the two retinoic acid responsive pathways are not simply redundant, but instead manifest a complex interplay.

In view of the related, but clearly distinct nature of these receptors, the identification of ligands which are more selective for the retinoid X receptor than is retinoic acid would be of great value in selectively controlling processes mediated by one or both of these retinoid receptor types.

Other information helpful in the understanding and practice of the present invention can be found in commonly assigned, co-pending United States Patent Application Serial Nos. 108,471, filed October 20, 1987 (now issued as United States Patent Number 5,071,773); 276,536, filed November 30, 1988 (now issued as United

States Patent Number 4,981,784); 325,240, filed March 17, 1989; 370,407, filed June 22, 1989; and 438,757, filed November 16, 1989, all of which are hereby incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have developed methods to modulate retinoid receptor mediated processes, employing high affinity, high specificity ligands for such receptors.

In a particular aspect of the present invention, there are provided ligands which are high affinity, high specificity ligands for retinoid receptors. Thus, in one aspect of the present invention, there are provided ligands which are more selective for the retinoid X receptor than is all-trans-retinoic acid. In another aspect of the present invention, we have discovered alternative ligands (other than all-trans-retinoic acid) which are capable of inducing retinoic acid receptor mediated processes.

In yet another aspect of the present invention, we have developed methods for the preparation of such retinoid receptor ligands from readily available retinoid compounds.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a transactivation profile of various HPLC fractions obtained from retinoic acid (RA)-treated S2 cells.

Figure 2a is a comparison of the transactivation profile of all-trans-retinoic acid (RA) on RAR-alpha and RXR-alpha.

Figure 2b is a similar comparison to that shown in Figure 2a, employing HPLC fraction 18 (instead of RA).

Figure 3 presents several activation profiles for analysis of RXR-alpha or RAR-alpha activation by various retinoic acid isomers. Panel a. represents experiments done in insect S2 cells, while panels b. and c. represent experiments done in mammalian CV-1 cells. In the figure, closed circles are used to designate 9-cis-retinoic acid, open circles are used for all-trans-retinoic acid, open triangles are used for 13-cis-retinoic acid and open squares are used for 11-cis-retinoic acid.

Figure 4 presents the results of saturation binding analysis of 9-cis-retinoic acid. Cell extracts were incubated with increasing concentrations of tritiated retinoid in the absence (total binding) or presence (non-specific binding) of 200-fold excess non-tritiated retinoid. Non-specific binding was subtracted from total binding and plotted as specific binding. The data shown in Figure 4a represent specific [³H]-9-cis-retinoic acid binding to RXR α (closed circles) or mock (open circles) extracts; or specific [³H]-all-trans-retinoic acid binding to RXR α (open squares).

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Figure 4b presents a Scatchard analysis, wherein specific 9-cis-retinoic acid binding to RXR α in (a) was transformed by Scatchard analysis and plotted. Linear regression yielded a $K_d = 11.7$ nM ($r=0.86$).

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Figure 5 presents a DNA-cellulose column profile of radiolabelled 9-cis-retinoic acid bound to baculovirus expressed RXR. In Figure 5a, sample cell extracts containing RXR α protein were labelled with 10 nM [³H]-9-cis-retinoic acid in the absence (open squares) or presence (open circles) of 200-fold excess non-radioactive 9-cis-retinoic acid, and then applied to the DNA-cellulose

column. Fall-through radioactivity was monitored until a consistent baseline was established. DNA-binding components were then eluted with a linear salt gradient. The peak radioactive fractions (labelled 1-15) were then 5 subjected to immunoblot analysis using an hRXR α -specific antisera. The peak radioactive fraction (indicated by an arrow) co-migrated exactly with the peak amount of RXR α -specific protein.

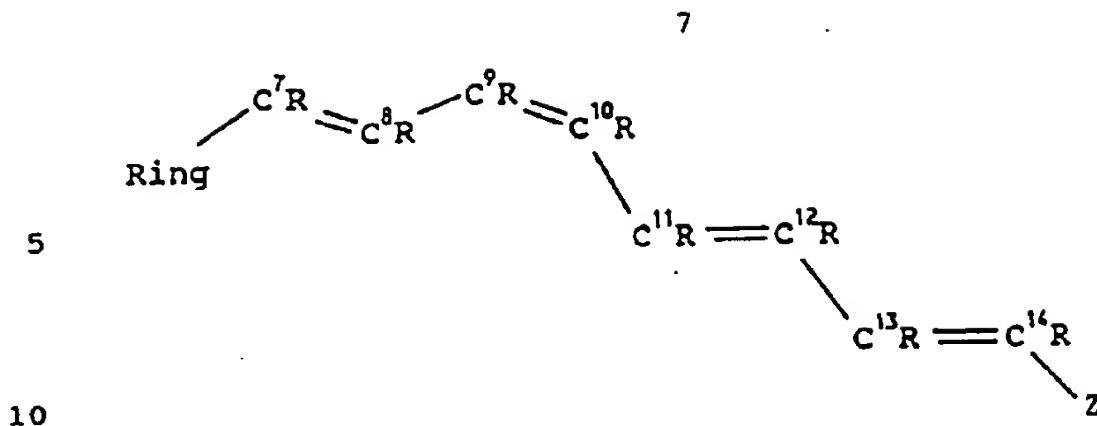
10 In Figure 5b, the peak radioactive fraction of the DNA-cellulose column is shown to contain 9-cis-retinoic acid. The peak fraction (arrow in (a)) was extracted and analyzed on a C₁₈ column developed with mobile phase G. As shown, 0.95% of the extracted radioactivity co-elutes with 15 authentic 9-cis-retinoic acid (absorbance peak).

Figure 6 is a comparison of the transactivation profile for RXR-alpha in the presence of 9-cis-retinoic acid employing a luciferase reporter containing the 20 retinoid response element derived from either the apolipoprotein A1 gene (APOA13) or cellular retinol binding protein, type II (CRBPII).

DETAILED DESCRIPTION OF THE INVENTION

25

In accordance with the present invention, there is provided a method for modulating process(es) mediated by retinoid receptors, said method comprising conducting said process(es) in the presence of at least one compound of the 30 structure:



Structure A

wherein:

15 unsaturation between carbon atoms C⁹ and C¹⁰
has a cis configuration, and one or both sites of
unsaturation between carbon atoms C¹¹ through C¹⁴
optionally have a cis configuration;

"Ring" is a cyclic moiety, optionally having one or more substituents thereon;

2 is selected from carboxyl (-COOH), carboxaldehyde (-COH), hydroxyalkyl $[-(CR')_n-OH]$, wherein each R' is independently selected from hydrogen or a lower alkyl and n falls in the range of 1 up to about 4, thioalkyl $[-(CR')_n-SH]$, wherein R' and n are as defined above, hydroxyalkyl phosphate $[-(CR')_n-OP(OM)_3]$, wherein R' and n are as defined above and M is hydrogen, lower alkyl or a cationic species such as Na^+ .

30 Li⁺, K⁺, and the like], alkyl ether of a hydroxyalkyl group $[-(CR')_n-OR'$, wherein R' and n are as defined above], alkyl thioether of a thioalkyl group $[-(CR')_n-SR'$, wherein R' and n

35 are as defined above], esters of hydroxyalkyl groups $[-(CR'_2)_n-O-CO-R']$, wherein R' and n are as defined above], thioesters of hydroxyalkyl group $[-(CR'_2)_n-O-CS-R']$, wherein R' and n are as defined

above], esters of thioalkyl groups $[-(CR')_n-S-CO-R'$, wherein R' and n are as defined above], thioesters of thioalkyl groups $[-(CR')_n-S-CS-R'$, wherein R' and n are as defined

above], aminoalkyl $[-(CR'_2)_n-NR'_2]$, wherein R' and n are as defined above], N-acyl aminoalkyl $[-(CR'_2)_n-NR'-CO-R'']$, wherein R' and n are as defined above and R'' is a lower alkyl or benzyl], carbamate $[-(CR'_2)_n-NR'-CO-OR']$ or $-(CR'_2)_n-O-CO-NR'_2$, wherein R' and n are as defined above], and the like; and

~~each R is independently selected from H,~~
~~halogen, alkyl, aryl, hydroxy, thiol, alkoxy,~~
10 ~~thialkoxy, amino, or any of the Z substituents,~~
~~and the like; or~~

any two or more of the R groups can be linked to one another to form one or more ring structures.

15 Exemplary R groups in the latter situation are selected from alkylene, oxyalkylene, thioalkylene, and the like.

As employed herein, the term "modulate" refers to the ability of a ligand for a member of the steroid/thyroid superfamily to induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control.

As employed herein, the phrase "processes
mediated by retinoid receptors" refers to biological,
physiological, endocrinological, and other bodily processes
which are mediated by receptor or receptor combinations
which are responsive to natural or synthetic retinoids, or
natural or synthetic compounds as defined herein (referred
to herein as "rexoids" because of the ability of many of
the compounds described herein to selectively activate
retinoid X receptors). Modulation of such processes can be
accomplished *in vitro* or *in vivo*. *In vivo* modulation can
be carried out in a wide range of subjects, such as, for
example, humans, rodents, sheep, pigs, cows, and the like.

Exemplary receptors which are responsive to retinoids, and natural or synthetic compounds as defined herein (i.e., "rexoids"), include retinoic acid receptor-alpha, retinoic acid receptor-beta, retinoic acid receptor-gamma, and splicing variants encoded by the genes for such receptors; retinoid X receptor-alpha, retinoid X receptor-beta, retinoid X receptor-gamma, and splicing variants encoded by the genes for such receptors; as well as various combinations thereof (i.e., homodimers, 10 homotrimers, heterodimers, heterotrimers, and the like), including combinations of such receptors with other members of the steroid/thyroid superfamily of receptors with which the retinoid receptors may interact by forming heterodimers, heterotrimers, and higher heteromultimers. 15 For example, the retinoic acid receptor-alpha may form a heterodimer with retinoid X receptor-alpha, the retinoic acid receptor-beta may form a heterodimer with retinoid X receptor-alpha, retinoic acid receptor-gamma may form a heterodimer with retinoid X receptor-alpha, retinoid X 20 receptor-alpha may form a heterodimer with thyroid receptor, retinoid X receptor-beta may form a heterodimer with vitamin D receptor, retinoid X receptor-gamma may form a heterodimer with retinoic acid receptor-alpha, and the like.

25

As employed herein, the phrase "members of the steroid/thyroid superfamily of receptors" (also known as "nuclear receptors" or "intracellular receptors") refers to hormone binding proteins that operate as ligand-dependent transcription factors, including identified members of the steroid/thyroid superfamily of receptors for which specific ligands have not yet been identified (referred to hereinafter as "orphan receptors"). These hormone binding proteins have the intrinsic ability to bind to specific DNA 30 sequences. Following binding, the transcriptional activity 35 of target gene (i.e., a gene associated with the specific DNA sequence) is modulated as a function of the ligand

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bound to the receptor.

The DNA-binding domains of all of these nuclear receptors are related, consisting of 66-68 amino acid residues, and possessing about 20 invariant amino acid residues, including nine cysteines.

A member of the superfamily can be identified as a protein which contains the above-mentioned invariant amino acid residues, which are part of the DNA-binding domain of such known steroid receptors as the human glucocorticoid receptor (amino acids 421-486), the estrogen receptor (amino acids 185-250), the mineralocorticoid receptor (amino acids 603-668), the human retinoic acid receptor (amino acids 88-153). The highly conserved amino acids of the DNA-binding domain of members of the superfamily are as follows:

Cys - X - X - Cys - X - X - Asp* - X -
20 Ala* - X - Gly* - X - Tyr* - X - X -
X - X - Cys - X - X - Cys - Lys* -
X - Phe - Phe - X - Arg* - X - X - X -
X - X - X - X - X - (X - X -) Cys -
X - X - X - X - X - (X - X - X -) Cys -
25 X - X - X - Lys - X - X - Arg - X - X -
Cys - X - X - Cys - Arg* - X - X -
Lys* - Cys - X - X - X - Gly* - Met
(SEQ ID No 1);

30 wherein X designates non-conserved amino acids within the DNA-binding domain; the amino acid residues denoted with an asterisk are residues that are almost universally conserved, but for which variations have been found in some identified hormone receptors; and the residues enclosed in 35 parenthesis are optional residues (thus, the DNA-binding domain is a minimum of 66 amino acids in length, but can contain several additional residues).

Exemplary members of the steroid/thyroid superfamily of receptors include steroid receptors such as glucocorticoid receptor, mineralocorticoid receptor, progesterone receptor, androgen receptor, vitamin D₃ receptor, and the like; plus retinoid receptors, such as RAR α , RAR β , RAR γ , and the like, plus RXR α , RXR β , RXR γ , and the like; thyroid receptors, such as TR α , TR β , and the like; as well as other gene products which, by their structure and properties, are considered to be members of the superfamily, as defined hereinabove. Examples of orphan receptors include HNF4 [see, for example, Sladek et al., in *Genes & Development* 4: 2353-2365 (1990)], the COUP family of receptors [see, for example, Miyajima et al., in *Nucleic Acids Research* 16: 11057-11074 (1988), Wang et al., in *Nature* 340: 163-166 (1989)], COUP-like receptors and COUP homologs, such as those described by Mlodzik et al., in *Cell* 60: 211-224 (1990) and Ladias et al., in *Science* 251: 561-565 (1991), the ultraspiracle receptor [see, for example, Oro et al., in *Nature* 347: 298-301 (1990)], and the like.

Processes capable of being modulated by retinoid receptors, in accordance with the present invention, include *in vitro* cellular differentiation and proliferation, *in vitro* proliferation of melanoma cell lines, *in vitro* differentiation of mouse teratocarcinoma cells (F9 cells), *in vitro* differentiation of human epidermal keratinocytes, limb morphogenesis, regulation of cellular retinol binding protein (CRBP), and the like. As readily recognized by those of skill in the art, the availability of ligands for the retinoid X receptor makes it possible, for the first time, to carry out assays for the identification of antagonists for said receptor.

35 Processes capable of being modulated by retinoid receptors, in accordance with the present invention, also include the *in vivo* modulation of lipid metabolism, *in vivo*

modulation of skin-related processes (e.g., acne, aging, wrinkling, skin cancer, and the like), in vivo modulation of malignant cell development, such as occurs, for example, in acute promyelocytic leukemia, testicular cancer, lung 5 cancer, and the like. The ability of compounds of the invention to modulate such processes is evidenced in a number of ways. See, for example, Figure 6 where the ability of RXR-alpha, in the presence of ligand therefor (e.g., 9-cis-retinoic acid) is shown to exert a strong 10 effect on the expression of genes under the control of regulatory elements of apolipoprotein AI. Similarly, studies with model systems for a variety of disease states (e.g., differentiation of HL60 cells as a model for acute promyelocytic leukemia, proliferation of melanoma cell 15 lines as a model for skin cancer, differentiation of keratinocytes as a model for non-malignant skin disorders, and the like), as set forth in the Examples, demonstrate the ability of retinoid receptors, in the presence of ligand therefor, e.g., 9-cis-retinoic acid, to exert a 20 strong effect on such disease states. Such in vivo applications of the invention process may allow the modulation of various biological processes with reduced occurrence of undesirable side effects, and the like.

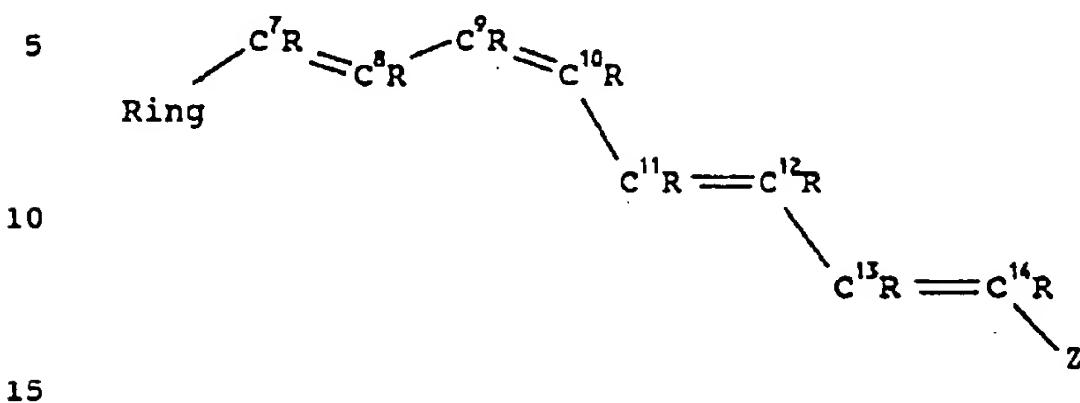
25 In vivo applications of the invention process(es) (and compositions) can be employed with a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

30 As employed herein, the term "alkyl" refers to "lower alkyl", i.e., alkyl moieties having in the range of 1 up to about 4 carbon atoms, i.e., methyl groups, ethyl groups, propyl groups, isopropyl groups, normal-butyl groups, isobutyl groups, sec-butyl groups, tert-butyl 35 groups, and the like.

cyclic moieties contemplated as part of the compounds employed in the practice of the present invention include 5-, 6-, and 7-membered carbocyclic, heterocyclic aromatic or heteroaromatic rings. Included in this definition, for example, are optionally substituted saturated, mono-unsaturated or polyunsaturated carbocyclic species, such as, for example, cyclopentane, cyclopentene, cyclohexane, cyclohex-2-ene, cyclohex-3-ene, cyclohex-4-ene, and cyclohex-5-ene isomers, and 2,4-, 2,5-, 10 and 3,5-cyclohexadiene variants thereof. Examples of heterocyclic species contemplated as part of the compounds employed in the practice of the present invention include dihydrofuran, tetrahydrofuran, dihydrothiophene, tetrahydrothiophene, dihydropyran, tetrahydropyran, 15 dihydrothiopyran, tetrahydrothiopyran, piperidine, pyrrolidine, and the like, as well as derivatives thereof. Examples of aromatic or heteroaromatic species contemplated as part of the rexoid compounds of the present invention include phenyl, tolyl, xylyl, mesityl, benzyl, pyridyl, 20 thiophenyl, furanyl, and the like, as well as derivatives thereof.

Preferred cyclic moieties are typically geminally di-substituted, mono-unsaturated species. Presently preferred geminally di-substituted, mono-unsaturated cyclic moieties are the 1,1,5-trisubstituted cyclohex-5-ene structure of naturally occurring retinoic acid (i.e., the ring structure of β -ionone; the position of the substituents on the ring are designated employing the 30 traditional retinoic acid numbering convention for the ring structure of β -ionone), as well as the 1,1,4,5-tri-substituted cyclohex-5-ene structure provided by hydroxy- or keto-substituted derivatives of the traditional β -ionone structure.

Compounds contemplated for use in the practice of the present invention include compounds having the structure:

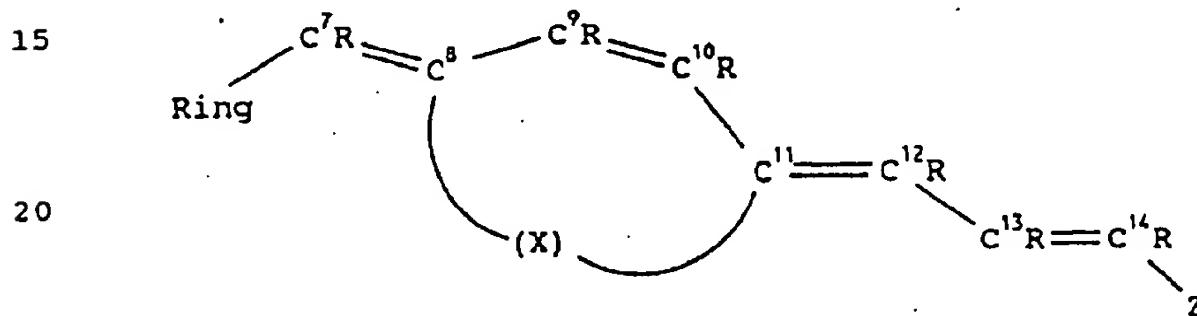


wherein:

- unsaturation between carbon atoms C^9 and C^{10}
has a cis configuration, and one or both sites of unsaturation between carbon atoms C^{11} through C^{14} optionally have a cis configuration;
- "Ring" is a cyclic moiety;
- Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, carbamate, and the like; and
- R on each of C^7 , C^8 , C^9 , C^{10} , C^{11} , C^{12} , C^{13} , or C^{14} is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents; or
any two or more of the R groups can be linked to one another to form one or more ring structures.
- Presently preferred compounds which are contemplated by the above generic structure include

9-cis-retinoic acid, as well as novel derivatives thereof such as 9-phenyl-9-cis-retinoic acid, 4-hydroxy-9-cis-retinoic acid, 4-keto-9-cis-retinoic acid, and the like.

5 In another preferred embodiment of the present invention, the substituents on C⁹ and C¹³ are methyl; in yet another preferred embodiment, the substituents on two or more of the side chain carbons (i.e., C⁷, C⁸, C⁹, C¹⁰, C¹¹, C¹², C¹³, or C¹⁴) can be linked together to form a ring structure. For example, the substituents on C⁸ and C¹¹ can be linked together to form a structure having a constrained 10 9-cis double bond (i.e., a 9-cis locked rexoid derivative), as follows:



25

Structure I

wherein:

X is -[(CR₂)_x-X'-(CR₂)_y]-,

X' is selected from -O-, carbonyl (>CO),

30 -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), -NR"-, or -CR₂-,

R, Ring and Z are as defined above,

35 R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl (-CO-O-alkyl);

x is 0, 1 or 2,

y is 0, 1, or 2, and

x + y ≤ 2.

Such compounds include cyclopentene derivatives,
40 cyclohexene derivatives, cycloheptene derivatives,

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dihydrofuran derivatives, dihydropyrrole derivatives, and the like, wherein the cyclic structure linking C⁸ and C¹¹ serves to prevent isomerization of the cis double bond between C⁹ and C¹⁰.

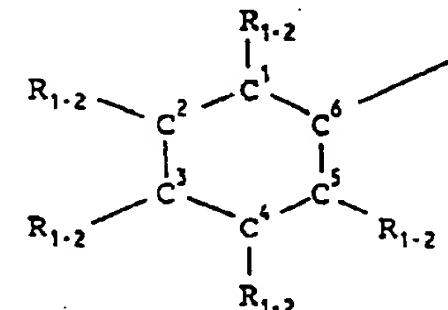
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Especially preferred derivatives of structure I are those where Z is a carboxyl group, and Ring is a β -ionone-like species having the structure:

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β -ionone ring structure

wherein:

each R is independently defined as provided above;

any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-; wherein R" is as defined above; and

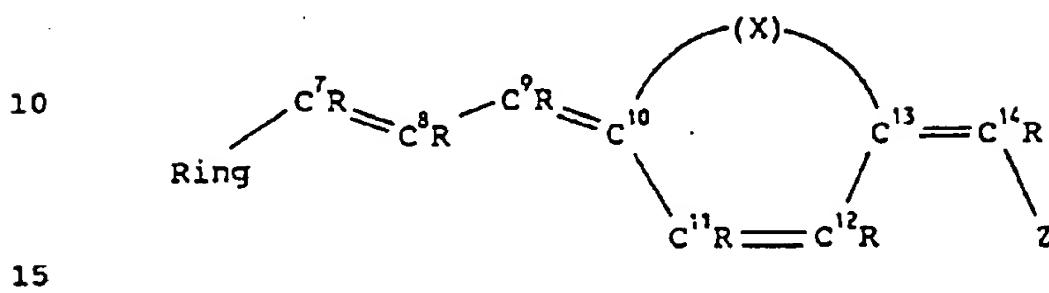
said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer; the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

Especially preferred species for use in the practice of the present invention are derivatives of structure I where Z is a carboxyl group, and Ring is a 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-tetrasubstituted cyclohex-5-ene structure.

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Similarly, the substituents on C¹⁰ and C¹³ can be linked together to form a structure having a constrained 9, 11-di-cis configuration (i.e., a 9-cis locked rexoid derivative), as follows:

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Structure II

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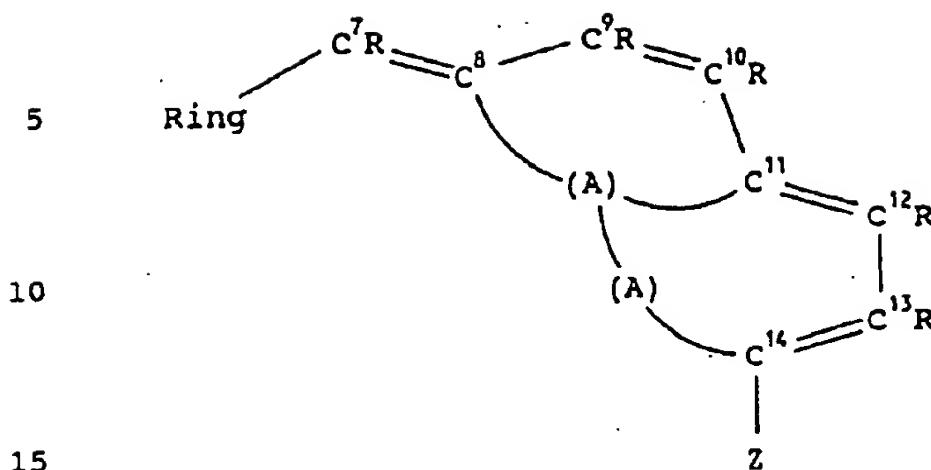
wherein:

X, X', R, R'', Z, Ring, x and y are as defined above.

25 Such compounds include cyclopentene derivatives, cyclohexene derivatives, cycloheptene derivatives, dihydrofuran derivatives, dihydropyrrole derivatives, and the like, wherein the cyclic structure linking C¹⁰ and C¹³ serves to hinder isomerization of the cis double bond 30 between C⁹ and C¹⁰, and prevent isomerization of the cis double bond between C¹¹ and C¹².

Especially preferred derivatives of Structure II are those where Z is a carboxyl group, and the Ring is a 35 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-tetrasubstituted cyclohex-5-ene structure.

Similarly, at least two of the substituents on C⁸, C¹¹, and/or C¹⁴ can be linked together to form a structure 40 having a constrained 9, 13-di-cis configuration (i.e., a 9-cis locked rexoid derivative), shown below as Structure III:

Structure III

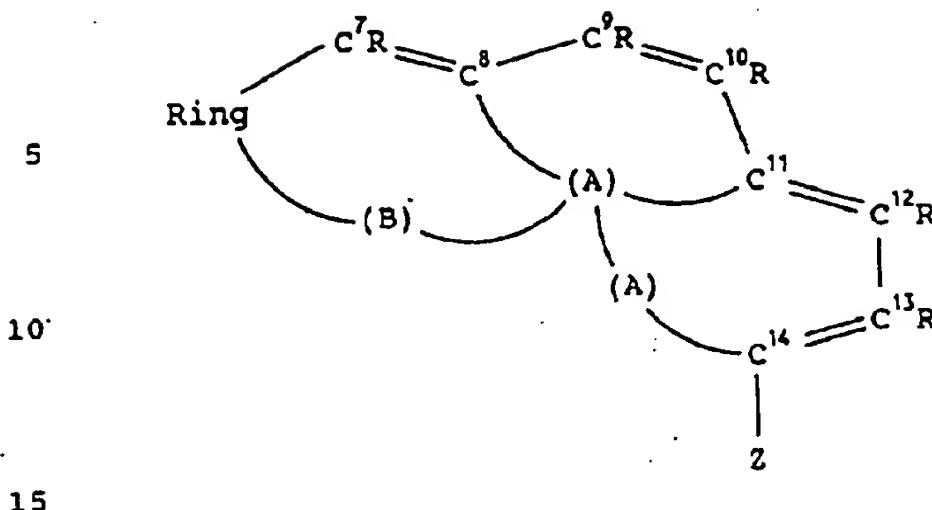
20

wherein:

one A is X and the other A is X', and X, X', R, R'', Z, Ring, x and y are as defined above. Those of skill in the art recognize that the junction between the two bridging groups (A) can only occur through an atom with a valence of three or four (i.e., through carbon or nitrogen), so as to accommodate the bonds required to link the fused rings together.

Similarly, at least two of the substituents on C⁸, C¹¹, and/or C¹⁴ can be linked together, and further linked to C⁵ of Ring, or to a substituent on C⁵ to form a structure having a constrained 9, 13-di-cis configuration (i.e., a 9-cis locked rexoid derivative), shown below as Structure IV:

19



15

Structure IV

20

wherein:

one A is X and the other A is X',

B is X', and

25

X, X', R, R'', Z, Ring, x and y are as defined above. As noted above with respect to Structure III, those of skill in the art recognize that the junction(s) between the bridging groups (A) and (B) can only occur through an atom with a valence of three or four (i.e., through carbon or nitrogen), so as to accommodate the bonds required to link the fused rings together.

Such compounds include cyclopentene derivatives,
35 cyclohexene derivatives, cycloheptene derivatives, dihydrofuran derivatives, dihydropyrrole derivatives, and the like, wherein the cyclic structures linking C⁸, C¹¹ and/or C¹³ serves to prevent isomerization of the cis double bonds at carbon 9 and carbon 13.

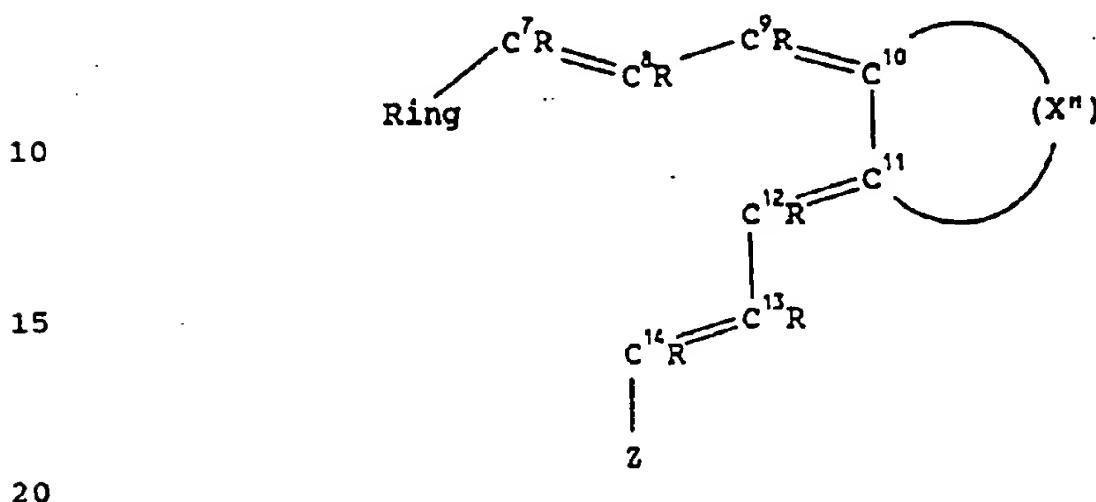
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Especially preferred derivatives of Structures III and IV are those where Z is a carboxyl group, and Ring is a 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-tetrasubstituted cyclohex-5-ene structure.

20

Similarly, the substituents on C¹⁰ and C¹¹ can be linked together to form a structure having a constrained 9-cis double bond (i.e., a 9-cis locked rexoid derivative), as follows:

5



10

15

20

Structure V

wherein:

25

X'' is $-[(CR_2)_a-X'--(CR_2)_b]-$,

X', R, R'', Ring and Z are as defined above,

a is 0, 1, 2, 3 or 4,

b is 0, 1, 2, 3, or 4, and

a + b is ≥ 2 , but ≤ 4 .

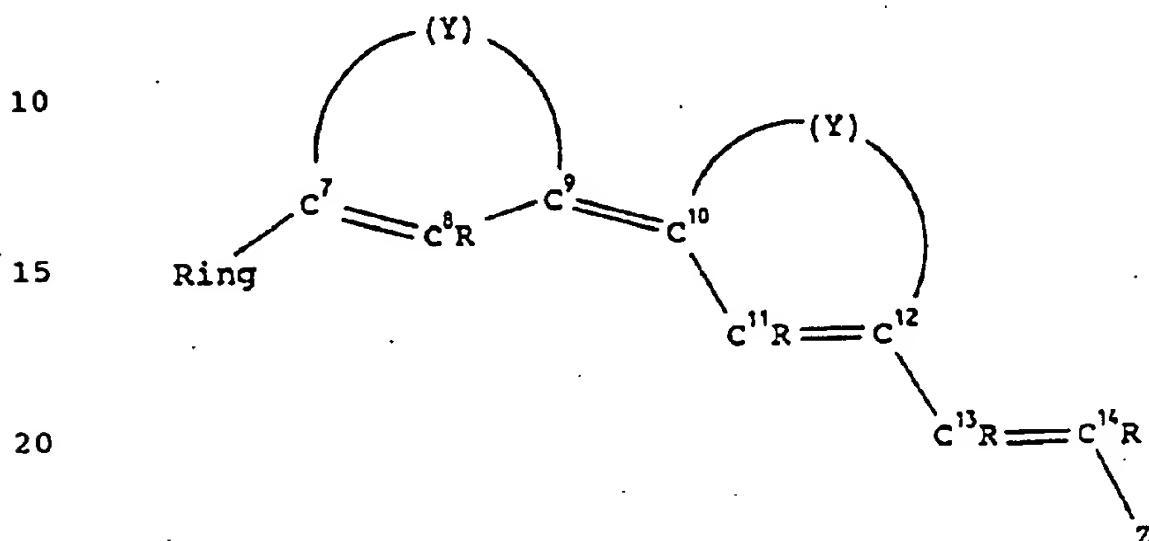
30

Such compounds include cyclopentene derivatives, cyclohexene derivatives, cycloheptene derivatives, dihydrofuran derivatives, dihydropyrrole derivatives, and the like, wherein the cyclic structure linking C¹⁰ and C¹¹ serves to prevent isomerization of the cis double bond between C⁹ and C¹⁰.

Especially preferred derivatives of Structure V are those where Z is a carboxyl group, and Ring is a 40 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-tetrasubstituted cyclohex-5-ene structure.

21

Similarly, the substituents on C⁷ and C⁹ can be linked together, and the substituents on C¹⁰ and C¹² can be linked together to form a structure having a constrained 9-cis double bond (i.e., a 9-cis locked rexoid derivative),
 5 as follows:



25

Structure VI

wherein:

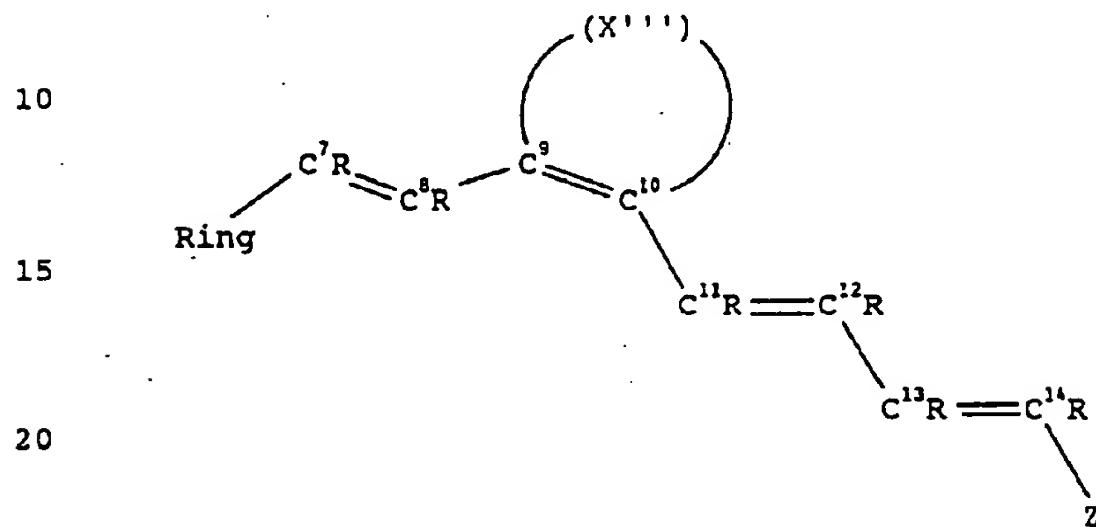
Y is $-[(CR_2)_c-X'-(CR_2)_d]-$,
 30 X', R, R'', Ring and Z are as defined above,
 c is 0, 1, 2 or 3,
 d is 0, 1, 2 or 3, and
 c + d ≥ 1, but ≤ 3.

35 Such compounds include cyclopentene derivatives, cyclohexene derivatives, cycloheptene derivatives, dihydrofuran derivatives, dihydropyrrole derivatives, and the like, wherein the cyclic structures linking C⁷ and C⁹, and C¹⁰ and C¹² serve to prevent isomerization of the cis 40 double bond between C⁹ and C¹⁰.

Especially preferred derivatives of Structure VI are those where Z is a carboxyl group, and Ring is a 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-

tetrasubstituted cyclohex-5-ene structure.

Similarly, the substituents on C⁹ and C¹⁰ can be linked together to form a structure having a constrained
 5 C-9 double bond (i.e., a 9-cis locked rexoid derivative),
 as follows:



25

Structure VII

wherein:

X''' is X" or an unsaturated linking group
 having the structure:

30

-[Q = CR - J]-,

wherein Q is -N= or -CR=, and J is -CR=CR-,
 -N=CR-, -CR=N-, -O-, -S-, or -NR"-,

35

thereby incorporating C⁹ and C¹⁰ of the rexoid
 compound into an aromatic (or pseudo-aromatic)
 ring, and

X', X", R, R", Ring, Z, a and b are as
 defined above.

40

Such compounds include cyclohexene derivatives,
 cycloheptene derivatives, benzene derivatives, pyridine
 derivatives, furan derivatives, thiophene derivatives,
 pyrrole derivatives, oxazole derivatives, thiazole

derivatives, imidazole derivatives, pyrazole derivatives, and the like, wherein the cyclic structure linking C⁹ and C¹⁰ serves to prevent isomerization of the C⁹-C¹⁰ double bond; however, rotation about the 8-9 and/or 10-11 single bonds can still occur.

Especially preferred derivatives of Structure VII are those where Z is a carboxyl group, and Ring is a 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-tetrasubstituted cyclohex-5-ene structure.

In addition to the structures set forth above, those of skill in the art can readily identify additional means to constrain the basic cis-configuration containing rexoid compounds employed in the practice of the present invention.

In accordance with a preferred embodiment of the present invention, the cyclic moiety has the β -ionone structure set forth above. Especially preferred are the 1,1,5-trisubstituted cyclohex-5-ene structure (characteristic of β -ionone) as well as the closely related 1,1,4,5-tetrasubstituted cyclohex-5-ene structure from which many rexoid compounds according to the present invention can be prepared.

In accordance with a particularly preferred embodiment of the present invention, the compounds employed in the invention process are selected from 9-cis-retinoic acid and derivatives thereof as contemplated by Structure A set forth above, as well as 9-cis-locked derivatives of retinoic acid as set forth in Structures I-VII above. Examples of specific compounds contemplated for use in the practice of the present invention are compounds wherein Z is carboxy, Ring is the 1,1,5-trisubstituted cyclohex-5-ene structure characteristic of β -ionone (or the closely related 1,1,4,5-tetrasubstituted cyclohex-5-ene), and having a side

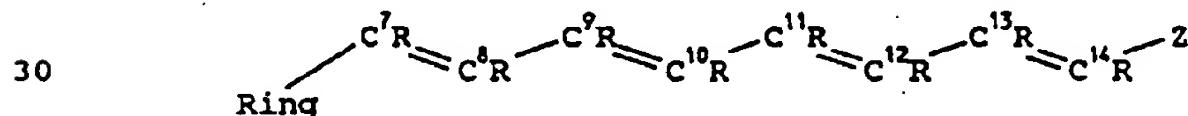
chain structure(s) as described above for Structures I-VII.

"Rexoid" derivatives as described above can be prepared employing a variety of synthetic methods, which 5 are readily available (and well known) to those of skill in the art. See, for example, the methods described in Chemistry and Biology of Synthetic Retinoids, Dawson and Okamura, eds., CRC Press, Inc. (1990), especially Chapter 4, by Ito (found at pages 78-97), and Chapter 9, by de Lera 10 et al. (found at pages 202-227) can readily be adapted for the preparation of the compounds described herein. The contents of this publication are hereby incorporated by reference herein. See also Asato et al., J. Am. Chem. Soc. 108: 5032 (1986); Sheves et al., J. Am. Chem. Soc. 108: 15 6440 (1986); Akita et al., J. Am. Chem. Soc. 102: 6370 (1980); Derguini and Nakanishi, Photobiochem. and Photobiophys. 13: 259 (1986), the entire contents of each of which is hereby incorporated by reference herein.

20 In accordance with another embodiment of the present invention, there is provided a method for modulating processes mediated by retinoid receptors, said method comprising conducting said process in the presence of:

25

(a) at least one compound of the structure:



wherein:

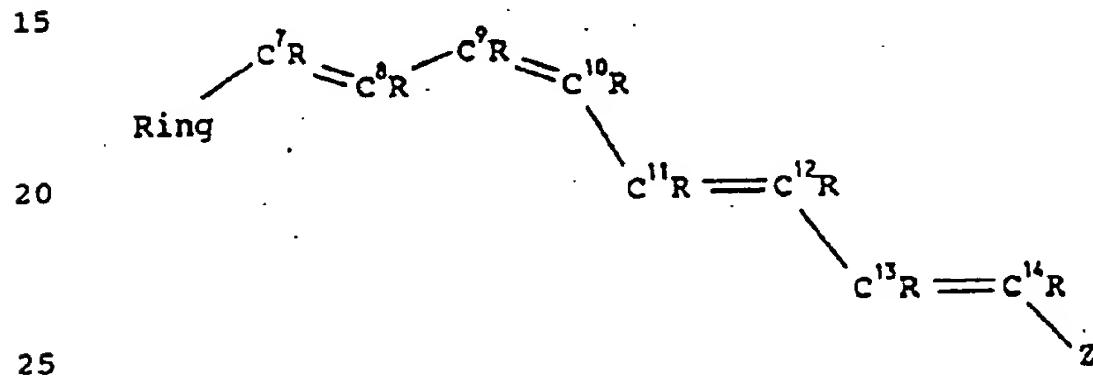
35 each site of unsaturation in the side chain comprising carbon atoms C⁷ through C¹⁴ has a trans configuration;

"Ring", Z, and R are as previously described, and

(b) a *cis/trans* isomerase capable of converting at least the 9-double bond from the *trans* configuration to the *cis*-configuration.

5 As employed herein, the term "*cis/trans* isomerase" refers to enzymes which promote a change of geometrical configuration at a double bond. Examples of such enzymes include maleate isomerase, maleylacetooacetate isomerase, retinal isomerase, maleylpyruvate isomerase, 10 linoleate isomerase, furylfuramide isomerase, and the like.

In accordance with yet another embodiment of the present invention, there is provided a method to produce compound(s) of the structure:



wherein:

15 unsaturation between carbon atoms C' and C'' has a *cis* configuration, and one or both sites of unsaturation between carbon atoms C'' through C'' 20 optionally have a *cis* configuration;

30 "Ring" is a cyclic moiety;

35 Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, carbamate, and the like; and

40 each R is independently selected from H,

halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

from the corresponding all-trans configuration material,
5 said method comprising contacting said all-trans configuration material with a cis/trans isomerase under isomerization conditions.

In accordance with still another embodiment of
10 the present invention, there are provided novel compositions comprising compound(s) of Structure A (excluding previously identified compounds such as retinoic acid as well as constrained compounds selected from Structures I - VII, as set forth above. Examples of such
15 compounds include 9-phenyl-9-cis-retinoic acid, 4-hydroxy-9-cis-retinoic acid, 4-keto-9-cis-retinoic acid, and the like. Presently preferred compounds are those wherein Z is carboxyl and Ring is a 1,1,5-trisubstituted cyclohex-5-ene structure or a 1,1,4,5-tetrasubstituted
20 cyclohex-5-ene structure.

The invention compounds can be employed for both in vitro and in vivo applications. For in vivo applications, the invention compounds can be incorporated
25 into a pharmaceutically acceptable formulation for administration. Those of skill in the art can readily determine suitable dosage levels when the invention compounds are so used.

30 As employed herein, the phrase "suitable dosage levels" refers to levels of compound sufficient to provide circulating concentrations high enough to effect activation of retinoid receptor(s). Such a concentration typically falls in the range of about 10 nM up to 2 μ M; with
35 concentrations in the range of about 100 nM up to 200 nM being preferred.

In accordance with a particular embodiment of the present invention, compositions comprising at least one 9-cis-retinoic acid-like compound (as described above), and a pharmaceutically acceptable carrier are contemplated.

Exemplary pharmaceutically acceptable carriers include carriers suitable for oral, intravenous, subcutaneous, intramuscular, intracutaneous, and the like administration. Administration in the form of creams, lotions, tablets, dispersible powders, granules, syrups, elixirs, sterile aqueous or non-aqueous solutions, suspensions or emulsions, and the like, is contemplated.

For the preparation of oral liquids, suitable carriers include emulsions, solutions, suspensions, syrups, and the like, optionally containing additives such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents, and the like.

For the preparation of fluids for parenteral administration, suitable carriers include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile water, or some other sterile injectable medium immediately before use.

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLESExample 1Identification of Compound(s) that Activate RXR

5

In order to ascertain if retinoic acid can be converted to a product that binds directly to RXR, thereby resulting in modulation of transcription, a strategy was developed to identify retinoic acid metabolites that might modulate the transcriptional properties of RXR. The identification of any such active metabolite would allow one to further determine whether this metabolite was capable of directly binding to the receptor protein.

15

Accordingly, the *Drosophila melanogaster* Schneider cell line (S2) was incubated with or without all-trans-retinoic acid (RA) for a period of 24 hours. Prior to the addition of retinoic acid, *Drosophila melanogaster* Schneider cell line (S2) cells were grown in Schneider *Drosophila* medium (GIBCO) supplemented with penicillin, streptomycin and 12% heat inactivated FCS (Irvine Scientific). One hundred tissue culture flasks (75 cm^2) were set up with 10^7 cells and 12 ml of medium/flask. Twenty four hours later, either all-trans-retinoic acid (or ethanol solvent control) was added to each flask to a final concentration of 5×10^{-6} M in reduced light conditions. Cells were harvested 24 hours later by centrifugation for 5 minutes at 800 g. Cells were washed twice with PBS and the resultant pellets were frozen at -80°C until extraction.

In parallel, CV-1 cells were set up on 64 tissue culture dishes (150 mm) at 2×10^6 cells and 25 ml of medium/dish. Cells were treated with retinoic acid and harvested as with the S2 cells except that the CV-1 cells (which are adherent) were washed in their dishes with PBS and scraped with a rubber policeman prior to centrifugation

and freezing.

Following incubation, the cell pellets were collected, organically extracted and chromatographically fractionated by HPLC. The various HPLC fractions were assayed for their ability to produce a ligand dependent increase in transcriptional activity mediated by RXR. This assay system involves transfecting cells with the cDNA for the RXR receptor and a luciferase reporter molecule which is under control of a promoter containing a RXR response element (RXRE) [see Mangelsdorf et al., Cell 66:555 (1991)]. The addition of a ligand capable of activating RXR results in an increase in luciferase activity.

Schneider cells, CV-1 cells and mouse tissues were extracted as described by C. Thaller and G. Eichele in Nature Vol. 327:625 (1987). Mouse tissue was used to determine if any RXR ligand is present *in vivo*. In the case of tissue extractions, 2.10^5 dpm internal standard [$11,12\text{-}^3\text{H}$]-all-trans-retinoic acid (New England Nuclear) or 9-cis-retinoic acid (generated by isomerization with light) were added to the homogenate. Extracts were fractionated on a Waters Novapak 300 mm C₁₈ analytical column at a flow rate of 1 ml min⁻¹. The mobile phase (G) was a 1:1 mixture of:

A [CH₃CN/CH₃OH/2% aqueous CH₃COOH (3:1:1)] and
E [CH₃CN/CH₃OH/2% aqueous CH₃COOH (11:3:10)].

Other mobile phases used have the following compositions:

C: CH₃CN/CH₃OH/H₂O/CH₃COOH (80:10:10:1),
H: mix CH₃OH/10 mM ammonium acetate (9:1) with equal volume of CH₃OH/10 mM ammonium acetate (3:1).

30

Methyl esters of retinoic acid isomers and/or metabolites contained in the HPLC fractions were generated as described in Wedden et al. [Meth. Enzymol. 190:201 (1990)]. Reference standards used were from Aldrich, Sigma or kindly provided by Hoffmann-LaRoche. Authentic 9-cis-retinol, 9-cis-retinoic acid and 9-cis-methylretinoate were either synthesized from 9-cis-retinal [see E.J. Corey et al., J. Am. Chem. Soc. 90:5616 (1968); C.D.B. Bridges & R.A. Alvares (Meth. Enzymol. 81:463 (1982))] or generated by photoisomerization of the all-trans isomer followed by fractionation of the resulting isomers by HPLC.

Photoisomerization of all-trans-retinoic acid is carried out employing standard isomerization techniques which are well known to those of skill in the art. For example, retinoic acid can be dissolved in a polar organic solvent such as ethanol, placed in a quartz cuvette, and irradiated with a variety of wavelengths of light (such as fluorescent light). Temperature at which irradiation is carried out is not critical; accordingly, irradiation can be carried out at room temperature. Irradiation time is also not critical; typical irradiation times are in the range of about 0.5-2 hours.

The various HPLC fractions were diluted 1:100 and assayed for their ability to modulate the transcriptional properties of RXR.

Cotransfection Assay in CV-1 Cells

30

A monkey kidney cell line, CV-1, was used in the cis-trans assay. Cells were transfected with two DNA transfection vectors. The trans-vector allowed efficient production of retinoid receptor (e.g., RAR or RXR) in these cells, which do not normally express these receptors. The cis-vector contains an easily assayable gene, in this case the firefly luciferase, coupled to a retinoid-responsive

promoter. Addition of retinoic acid or an appropriate synthetic retinoid results in the formation of a retinoid-receptor complex that activates the luciferase gene, causing light to be emitted from cell extracts. The level 5 of luciferase activity is directly proportional to the effectiveness of the retinoid-receptor complex in activating gene expression. This sensitive and reproducible cotransfection approach permits the identification of retinoids that interact with the 10 different receptor isoforms.

Cells were cultured in DMEM supplemented with 10% charcoal resin-stripped fetal bovine serum, and experiments were conducted in 96-well plates. The plasmids were 15 transiently transfected by the calcium phosphate method [Umesono and Evans, Cell 57:1139-1146 (1989); Berger et al., J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)] by using 10 ng of a pRS (Rous sarcoma virus promoter) receptor-expression plasmid vector, 50 ng of the reporter 20 luciferase (LUC) plasmid, 50 ng of pRS β -GAL (β -galactosidase) as an internal control, and 90 ng of carrier plasmid pGEM. Cells were transfected for 6 hours and then washed to remove the precipitate. The cells were then incubated for 36 hours with or without retinoid. 25 After the transfection, all subsequent steps were performed on a Beckman Biomek Automated Workstation. Cell extracts were prepared as described by Berger et al. supra, then assayed for luciferase and β -galactosidase activities. All determinations were performed in triplicate in two 30 independent experiments and were normalized for transfection efficiency by using β -galactosidase as the internal control. Retinoid activity was normalized relative to that of retinoic acid and is expressed as potency (EC50), which is the concentration of retinoid 35 required to produce 50% of the maximal observed response, and efficacy (%), which is the maximal response observed relative to that of retinoic acid at 10⁻⁵ M.

The receptor expression vectors used in the cotransfection assay have been described previously [pRShRAR- α : Giguere et al., *Nature* 330:624-629 (1987); pRShRAR- β and pRShRAR- γ : Ishikawa et al., *Mol. Endocrinol.*

5 4:837-844 (1990); retinoid X receptor-alpha (RXR- α) (see Mangelsdorf et al., in *Nature* 345: 224-229 (1990)], retinoid X receptor-beta (RXR- β) and retinoid X receptor-gamma (RXR- γ) (see Mangelsdorf et al., *Genes and Development* 6:329-344 (1992)). A basal reporter plasmid
10 Δ MTV-LUC [Hollenberg and Evans, *Cell* 55:899-906 (1988)] containing two copies of the TRE-palindromic response element 5'-TCAGGTCATGACCTGA-3' [SEQ ID No 2; see Umesono et al., *Nature* 336:262-265 (1988)] was used in all transfections for the retinoid receptors.

15

The bacterial expression vector for PET-8c-RAR- α used in the competitive binding assay has been reported [Yang et al., *Proc. Natl. Acad. Sci. USA* 88:3559-3563 (1991)]. Similar expression vectors employing the PET-8c
20 vector system [Studier et al., *Methods in Enzymology* 185:60-69 (1990)] were constructed for RAR- β and RAR- γ .

The transactivation profile of RXR-alpha with the various HPLC fractions containing various retinoic acid isomers and/or metabolites is shown in Figure 1. These data reveal two distinct regions of activity, one relatively early (fraction 7) and a second broader region of activity (fractions 16-21) that elutes considerably later. The all-trans-retinoic acid coelutes in fractions
25 20 and 21 (Figure 1) and is the major U.V. absorbing material present in the cell extracts. However, the activity profile demonstrates that, in addition to all-trans-retinoic acid, there are active components that must be derived from, or induced by, all-trans-retinoic
30 acid that activate RXR.
35

To identify potential compounds that would be as effective or more active than all-trans-retinoic acid, one must take into account not only the activity of the individual fractions, but also their concentrations. All 5 active fractions were therefore reassayed over a broad range of concentrations, taking into account the relative concentrations of the individual fractions. To determine the relative concentrations of the fractions, the following initial assumptions were made: 1) the active fractions are 10 retinoic acid metabolites and 2) the molar extinction coefficient of the various active fractions is relatively similar (i.e., within a factor of two). This assumption is supported by values reported in the literature for a large number of retinoids. A comparison of the transactivation 15 profile of all-trans-retinoic acid (i.e., fraction 20) on RAR-alpha and RXR-alpha is shown in Figure 2a. Although the maximal activation (i.e., efficacy) of RAR and RXR with retinoic acid is similar, RAR is more sensitive by a factor of approximately 10 fold (i.e., 10 fold more potent). In 20 contrast, analysis of the various fractions produced as described above demonstrates that fraction 18 is considerably more active on RXR than RAR (see Figure 2b). These data suggest that a metabolic product present in S2 cells pretreated with retinoic acid is a more potent 25 activator of the RXR subfamily than the RAR subfamily.

Example 2
Identification of 9-cis retinoic acid
as a transactivator of RXR

Two observations suggest that fraction 18 (peak X, see Fig. 1) is a cellular metabolite of all-trans-retinoic acid. First, extracts of Schneider cells grown in the absence of all-trans-retinoic acid do not exhibit peak 35 X. Second, when cells are exposed to all-trans-retinoic acid, X appears in a time-dependent fashion.

Therefore, to chemically identify X, fraction 18 was subjected to chemical derivatization, high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS). It was found that upon methylation 5 with diazomethane, the retention time of peak X shifts dramatically (i.e., from 10.2 minutes to 19.5 minutes under the HPLC conditions used). This indicates that the compound(s) corresponding to peak X has a free carboxyl group. When methylated X was analyzed by GC/MS, the 10 electron impact mode revealed that X gives rise to a molecular ion at m/z 314, corresponding to that of a retinoic acid methyl ester. This suggests that X is a stereoisomer of retinoic acid. To determine which isomer X represents, the retention time of X was compared with 15 that of 9-cis-, 11-cis- and 13-cis-retinoic acid. It was found that X coelutes with authentic 9-cis-retinoic acid. Furthermore, the methyl ester of X coelutes with 9-cis-methylretinoate, and when the methyl ester of X is reduced to the alcohol with lithium aluminum hydride, the resulting 20 product coelutes with authentic 9-cis-retinol.

For GC/MS analysis, methylated retinoic acid isomers were dissolved in hexane. The sample was injected via a falling needle injector (280°C) into a 30 m x 0.32 mm 25 fused silica DB5 capillary column (J+J scientific) inserted directly into the ion source of a VG Trio-1000 mass spectrometer operating in electron impact mode (70 eV). The sample was eluted with a temperature gradient (200-300°C, 10°C min⁻¹).

30

Finally, the mass spectrum of authentic 9-cis-retinoic acid methyl ester and that of methylated peak X are found to be identical. Taken together these analyses establish that peak X represents 9-cis-retinoic 35 acid. Although earlier work indicated the presence of 9-cis-retinol in fish liver, it was not clear whether 9-cis-retinoic acid existed in vivo (i.e., whether

9-cis-retinoic acid is a physiological compound). To find out if 9-cis-retinoic acid exists in vivo, mouse liver and kidney tissues were extracted. These tissues were selected because they contain a broad spectrum of retinoid metabolites and also express RXR. Prior to extraction, radiolabeled 9-cis-retinoic acid was added to the kidney homogenate to serve as an internal standard. Extracts were first fractionated on a reverse phase column (Waters Novo pak 300 mm C₁₈ analytical column at a flow rate of 1 ml/min) using mobile phase G.

Fractions from the kidney extracts containing radioactive internal standard were rechromatographed on a second C₁₈ column using mobile phase H. This procedure gave a small, but distinct absorbance peak which co-migrated with authentic 9-cis-retinoic acid.

Similarly, liver extract was fractionated on a reverse phase column and eluted with mobile phase G. However under the conditions employed, 9-cis-retinoic acid eluted with all-trans-retinol (which is abundantly present in the liver). To separate these two retinoids, this fraction was methylated with diazomethane and then reanalyzed by HPLC employing mobile phase C. This approach resulted in a distinct peak coeluting with the authentic methyl ester of 9-cis-retinoic acid.

To rule out the possibility that 9-cis-retinoic acid had formed during the extraction procedure from all-trans-retinoic acid, liver tissue homogenate was spiked with tritiated all-trans-retinoic acid. Subsequent HPLC fractionation revealed that 94% of the radioactivity still resided in all-trans-retinoic acid, approximately 5% in 13-cis-retinoic acid and 1% or less in 9-cis-retinoic acid. Based on peak area integration the concentrations of 9-cis-retinoic acid in liver and kidney are estimated to be

36

-4 ng, and ~4 ng, respectively, per g of wet weight. This indicates that endogenous 9-cis-retinoic acid is not formed from all-trans-retinoic acid during extraction. In conclusion, these experiments establish that 9-cis-retinoic acid is a naturally occurring retinoic acid isomer.

Example 3
Transactivation Profile of Retinoid
Isomers on RXR and RAR

10

The establishment that peak X represents a stereoisomer of all-trans-retinoic acid suggested that the various retinoid isomers may have different retinoid receptor activation profiles. To further analyze the ability of retinoic acid isomers to modulate the transcriptional properties of RXR-alpha and RAR-alpha, the four major photoisomers of all-trans-retinoic acid were identified and assayed for the ability to transactivate RXR and RAR. Figure 3 shows the dose response curves for 13-cis-, 11-cis-, 9-cis- and all-trans-retinoic acid for both RAR-alpha and RXR-alpha.

Of the four major isomers of retinoic acid, 9-cis-retinoic acid is seen to be the most potent and efficacious activator of RXR-alpha in both insect S2 cells (see Figure 3A) and mammalian CV-1 cells (see Figure 3B). The maximal response (EC₅₀ value) is 10^{-8} M and 5×10^{-8} M, respectively. The observed rank order of potency for the different isomers is the same in both cell lines. 9-cis-retinoic acid is approximately 40 fold more potent as an activator of RXR than 11-cis-, 13-cis- or all-trans-retinoic acid. These transactivation data strongly suggest that 9-cis-retinoic acid is an endogenous RXR-alpha activator.

35

In contrast, 9-cis-retinoic acid is equipotent to all-trans-retinoic acid as an activator of RAR-alpha

(Figure 3C). The EC₅₀ value for 9-cis-retinoic acid on RAR-alpha is 2×10^{-7} M. 9-cis-retinoic acid is the most potent RXR-alpha ligand to be tested to date.

5 Similarly, transactivation of other isoforms of RXR (i.e., RXR-beta, RXR-gamma) and RAR (i.e., RAR-beta, RAR-gamma) by 9-cis-retinoic acid was also examined. 9-cis-retinoic acid was also found to be a potent activator of these isoforms as well, as shown in Table 1:

10

Table 1

		EC ₅₀ * (nM)
15	<u>Receptor</u>	<u>All-trans-retinoic Acid</u> <u>9-cis-retinoic Acid</u>
	RAR- α	3861 \pm 13
	RAR- β	152 \pm 12
	RAR- γ	48 \pm 8
	RXR- α	1174 \pm 26
	RXR- β	1841 \pm 26
20	RXR- γ	1369 \pm 26
		254 \pm 19

*Meant SEM

25

Example 4

9-cis retinoic acid Binds Directly to RXRs

The ability of 9-cis-retinoic acid to transactivate RXR-alpha suggested testing to see whether 30 9-cis-retinoic acid was also capable of binding directly to RXRs. RXR-alpha was expressed in baculovirus and was shown to have biochemical properties that were identical to the mammalian expressed protein. The baculovirus expressed protein had a molecular weight of 51,000, reacted

specifically with RXR-alpha antibody and was capable of binding *in vitro* to DNA sequences that have been previously shown to be specific RXR response elements (i.e. CRBPII, see Mangelsdorf et al., *Cell* 66:555 (1991); apolipoprotein AI gene, see Rottman et al., *Mol. Cell Biol.* 11:3814 (1991)).

To characterize the ligand binding characteristics of 9-cis-retinoic acid to baculovirus-derived RXR, saturation binding analysis was carried out (see Figure 4). Radiolabelled 9-cis-retinoic acid binds specifically to RXR-alpha in a saturable manner. Scatchard analysis suggests a single high affinity binding site with a Kd value of 11.7 nM (see Figure 4b). Under identical binding conditions [³H]-all-trans-retinoic acid did not bind to RXR-alpha (see Figure 4a). In addition, 9-cis-retinoic acid was also capable of binding specifically to RAR-alpha as a high affinity ligand. 9-cis-retinoic acid did not bind to mock baculovirus extracts (i.e., control extracts from cells that do not express RXRs).

Similarly, binding studies were also carried out with other isoforms of RXR (i.e., RXR-beta, RXR-gamma), other isoforms of RAR (i.e., RAR-beta, RAR-gamma), and cellular retinoic acid binding protein (CRABP) with all-trans-retinoic acid and 9-cis-retinoic acid. While all-trans-retinoic acid is known to bind to each of these "receptors", 9-cis-retinoic acid was also found to bind to the other isoforms of retinoid receptors (but not to the cellular retinoic acid binding protein, CRABP), as shown in Table 2:

Table 2

Kd (nM)

<u>Receptor</u>	<u>All-trans-retinoic Acid</u>	<u>9-cis-retinoic Acid</u>
5 RAR- α	0.4	0.3
	0.4	0.2
	0.2	0.8
RXR- α	No binding	1.5
RXR- β	No binding	2.1
10 RXR- γ	No binding	1.9
CRABP	20	>100

The properties of many members of the steroid
15 hormone receptor superfamily have been characterized and
defined using DNA cellulose chromatography [see, for
example, Pike and Haussler, Proc. Natl. Acad. Sci. USA
76:5485 (1979) and Pike et al., J. Biol. Chem. 258:1289
(1983)]. Receptors, such as the VDR, have been shown in
20 the presence of their cognate ligand to bind to DNA-
cellulose [see, for example, Allegretto et al., J. Biol.
Chem. 262:1312 (1987)] with high affinity and the ligand-
receptor complex elutes with a salt gradient. A DNA-
25 cellulose column profile of the baculovirus expressed RXR
that had been prelabeled with [³H]-9-cis-retinoic acid is
shown in Figure 5. The two different profiles represent
1) the total amount of [³H]-9-cis-retinoic acid bound and
2) the level of binding that remains in the presence of
30 200-fold excess of cold (i.e. non-labeled 9-cis-retinoic
acid).

There is a peak of radioactivity (marked in the
Figure by an arrow) that elutes off the DNA-cellulose
column at 0.15 M KCl. This elution profile is similar to

40

that seen with RAR α in the presence of [3 H]-all-trans-retinoic acid. A 200 fold excess of cold ligand (i.e. non-specific) is capable of competing greater than 90% of the total radioactivity bound, demonstrating that the 5 radioactivity in the peak fractions is 9-cis-retinoic acid specifically bound to RXR.

The radioactivity eluted off the column was extracted with organic solvent and subjected to HPLC 10 analysis.

Inspection of Figure 5b makes it clear that the radioactivity bound to RXR co-chromatographs with authentic 9-cis-retinoic acid. This observation further confirms 15 that [3 H]-9-cis-retinoic acid is the species bound to RXR.

To demonstrate that the protein contained in the peak fractions is indeed RXR, these fractions (labelled 1-15 in Figure 5a) were subjected to immunoblot analysis 20 using an RXR α specific polyclonal antiserum (see Figure 5a, top). All fractions containing radioactivity display a distinct RXR α band at a M_r of 51,000. When a similar experiment was conducted with a baculovirus mock extract, no specific radioactivity was retained on the column. 25 Taken together, these data strongly suggest that 9-cis-retinoic acid is capable of binding specifically to RXR.

Protein samples were resuspended in 2X sample 30 buffer [Laemmeli, Nature Vol. 227:680 (1970)] and boiled for 5 minutes prior to loading onto a 9% SDS polyacrylamide gel. After electrophoretic separation the gels were electroblotted onto nitrocellulose membranes (Scheicher and Schuell) for 8 hours at 30 volts using a Hoeffer electro-35 transfer apparatus. Membranes were then incubated in 10% isopropanol, 10% acetic acid for 15 minutes, washed 5 minutes in deionized H₂O and 5 minutes in T-TBS buffer (10

mM Tris pH 7.5, 150 mM NaCl and 0.5% Triton X-100). The membranes were blocked in 5% nonfat milk in T-TBS for 1 hour. The remainder of the protocol was adapted from the Amersham ECL (Enhanced Chemiluminescence) Western blotting detection system kit. The primary antibody was a rabbit polyclonal serum raised against a synthetic peptide corresponding to amino acids 214-229 of hRXR α [Kliener et al., Proc. Natl. Acad. Sci. USA 89:1448-1452 (1992)]. The primary antiserum was diluted 1:5000 in T-TBS. The secondary antibody (Donkey anti rabbit IgG conjugated to horseradish peroxidase, Amersham) was used at a dilution of 1:2500.

Example 5

15 Effects of topical application of 9-cis-retinoic acid
 (compared with all-trans-retinoic acid)
 on horn-filled utriculus size in the Rhino Mouse

All-trans-retinoic acid is known to influence 20 cell differentiation and exert profound therapeutic benefits in the treatment of keratinization disorders [Elias et al., Arch. Dermatol. Vol. 117:160-180 (1981)]. Mezick et al. [see J. Invest. Derm. Vol. 83:110-113 (1984)] demonstrated that topical treatment of rhino mice (hr hr) 25 with all-trans-retinoic acid could reduce keratinized pilosebaceous structures (horn-filled utriculus). This animal test model was used to evaluate the "antikeratinizing" effects of 9-cis-retinoic acid. Results are summarized in Table 3:

Table 3

Pilosebaceous structure size (% red'n)

Vehicle Control	178 μm		
9-cis-retinoic acid,			
5 0.1%	52 μm	(-74%)	
0.01%	72 μm	(-64%)	
All-trans-retinoic acid,			
0.1%	44 μm	(-78%)	
0.01%	50 μm	(-75%)	

10

9-cis-retinoic acid reduced the mean utriculi diameter after 14 days of topical application. These results demonstrate that topical application of 9-cis-retinoic acid over a 14 day period can reduce keratinized pilosebaceous structures (horn-filled utriculus) in Rhino mouse skin. Reduction in the mean utriculi diameter by 9-cis-retinoic acid was comparable to that observed with all-trans-retinoic acid.

Example 6
Effects of 9-cis-retinoic acid (compared with
all-trans-retinoic acid) on differentiation of HL60 cells

25

Retinoids are known to differentiate human promyelocytic leukemia cells. Differentiation of HL60 cells (a model system for promyelocytic leukemia) can be assessed by Nitro Blue Tetrazolium (NBT) dye reduction (superoxide anion generation) and by measurement of up-regulation of the gene encoding the β subunit of the leukocyte adherence receptor, CD18 (J.B.C. vol. 263 No. 27, pp. 13863-13867).

35 The EC-50 for 9-cis-retinoic acid-mediated differentiation, as determined by NBT after 6 days treatment, was 0.2 μM compared to 2 μM for

all-trans-retinoic acid. Maximal effects (efficacies) were comparable, and CD18 was up-regulated by both ligands. Alpha-interferon potentiated both all-trans-retinoic acid and 9-cis-retinoic acid-mediated differentiation, as 5 determined by NBT.

HL60R cells have been shown to be resistant to differentiation by all-trans-retinoic acid, probably related to a mutation in the retinoic acid receptor-alpha 10 gene. This cell line was found to be resistant to differentiation (NBT) by both all-trans-retinoic acid and 9-cis-retinoic acid at concentrations up to 10 μ M.

9-cis-retinoic acid effects differentiation of 15 HL60 cells as evidenced by NBT and up-regulation of CD18. Compared with all-trans retinoic acid, 9-cis retinoic acid is more potent with similar efficacy.

Example 7

20 Effects of 9-cis-retinoic acid (compared with
 all-trans-retinoic acid) on in vitro
 proliferation of melanoma cell lines

All-trans-retinoic acid and several synthetic 25 analogs (retinoids) have been shown to prevent the development of benign and malignant, chemically induced epithelial tumors in vivo [Sporn et al., Fed. Proc. Vol. 35:1332-1338 (1976)]. Lotan et al. (J. Natl. Cancer, Vol. 60:1035-1041, 1978) found that all-trans-retinoic acid 30 inhibited the growth of several tumor cell lines in vitro. In view of these earlier findings, it was of interest to evaluate the growth inhibitory properties of 9-cis-retinoic acid.

35 9-cis-retinoic acid inhibited the growth of the murine melanoma cell line Clone M3 in a concentration

dependent manner, as follows:

<u>% Growth inhibition (Conc added)</u>		
	<u>1 μM</u>	<u>0.01 μM</u>
5 9-cis-retinoic acid	-85%	-49%
all-trans-retinoic acid	-94%	-48%

Similarly, 9-cis retinoic acid inhibited the growth of the human primary metastatic melanoma cell line 10 c81-46c in a concentration dependent manner.

<u>% Growth inhibition (Conc added)</u>		
	<u>1 μM</u>	<u>0.01 μM</u>
9-cis-retinoic acid	-45%	-28%
15 all-trans-retinoic acid	-44%	-17%

In summary, 9-cis-retinoic acid has been shown to inhibit the in vitro proliferation of murine melanoma cell line Clone M3 and human metastatic melanoma cell line 20 c81-46c in a concentration dependent manner. 9-cis-retinoic acid has an equal inhibitory effect on these cells as compared to all-trans-retinoic acid.

Example 8

Effects of 9-cis-retinoic acid (compared with all-trans-retinoic acid) on differentiation of F9 cells

Retinoids are known to differentiate mouse teratocarcinoma cells (F9). Differentiation of F9 cells is 30 specifically associated with irreversible changes in morphology and induction of the biochemical marker alkaline phosphatase (ALP) and tissue plasminogen activator (tPA) (Biochem. J. Vol. 274:673-678).

Both all-trans-retinoic acid and 9-cis-retinoic acid induced differentiation of F9 cells into partial endoderm-like cells as indicated by irreversible changes in

cellular morphology. All-trans-retinoic acid was 40 times more potent than 9-cis-retinoic acid in inducing ALP, maximal responses were similar.

5 The response of tissue plasminogen activator factor was less for 9-cis-retinoic acid than for all-trans-retinoic acid. At a concentration of 1 μ M of 9-cis-retinoic acid (or all-trans-retinoic acid), increased cellular activities of tPA by 0.48 ± 0.05 and 0.80 ± 0.08 , 10 respectively were observed. This effect was concentration-dependent.

In summary, 9-cis-retinoic acid promoted differentiation of F9 cells as evidenced by changes in 15 morphology and marker enzyme activities. Compared with all-trans-retinoic acid, 9-cis-retinoic acid was less potent with regard to both enzyme markers. Efficacy was comparable with ALP but indeterminate for tPA.

20

Example 9

Effects of 9-cis-retinoic acid (compared with all-trans-retinoic acid) on differentiation of keratinocytes

Retinoids are known to inhibit squamous cell 25 differentiation of cultured normal human epidermal keratinocytes (NHEK534 cell line), as judged by morphological alterations and inhibition of induction of transglutaminase (Type I) (J. Biol. Chem. Vol. 261:15097, 1986; Lab. Invest. Vol. 56:654, 1987).

30

Both all-trans-retinoic acid and 9-cis-retinoic acid inhibited squamous cell differentiation in a concentration dependent manner as judged by morphological changes and by transglutaminase activity. The EC50s for 35 inhibition of differentiation by all-trans-retinoic acid and 9-cis-retinoic acid were identical (20 ± 2.8 nM). 9-cis retinoic acid and all-trans-retinoic acid EC50s and

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potencies were nearly identical for effects on transglutaminase activities.

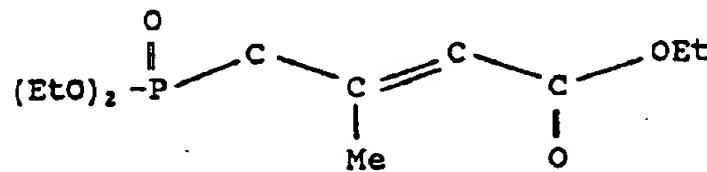
In summary, like all-trans-retinoic acid,
5 9-cis-retinoic acid inhibits morphological differentiation
of NHEK534 cells and induction of transglutaminase
activity.

Example 10

Synthesis of 9-phenyl-9-cis-retinoic acid

To a solution of 44 mg (0.10 mmole) of the following phosphonate reagent:

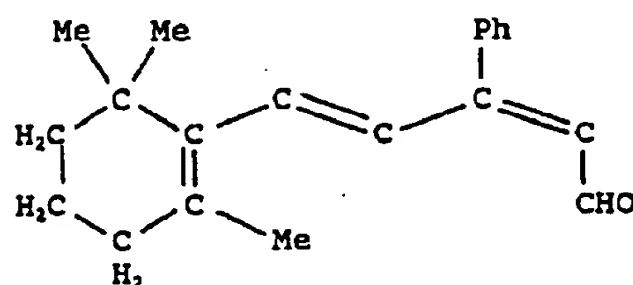
15



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in THF (0.5 ml) at room temperature was added NaH (60% in oil, 5 mg; 0.13 mmole) and the mixture stirred at that temperature for 10 minutes. To this, 26 mg (0.08 mmole) of the aldehyde:

30



35

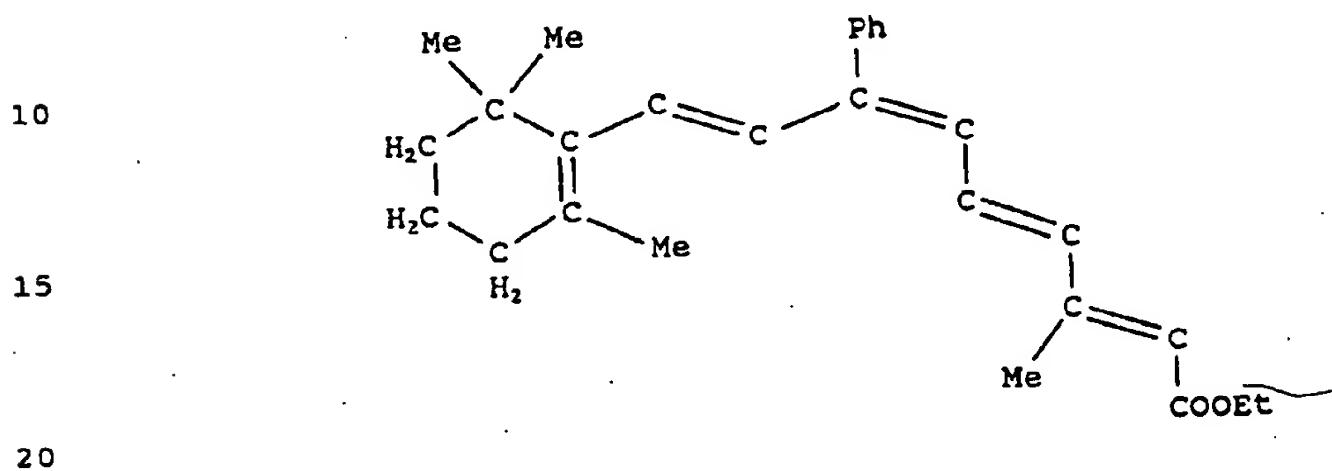
in THF (0.5 ml) was added at room temperature and the mixture allowed to stir for 30 minutes. Aqueous workup in the usual manner (NH_4Cl (aq), H_2O , brine, MgSO_4) gave a mixture of 9-phenyl-9-cis ester and 9-phenyl-9,13-dicis-

47

ester (30 mg, 92%) (the calculated ratio of 9-cis : 9,13-dicis = 4:1).

5

ethyl ester of 9-phenyl-9-cis-retinoic acid:

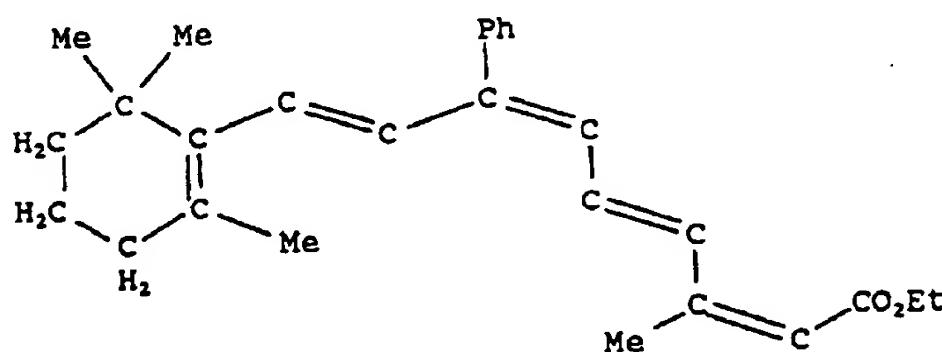


ethyl ester of 9-phenyl-9,13-dicis-retinoic acid:

25

30

35



To a mixture of 9-cis and 9,13-dicis ester (20 mg, 0.05 mmole) in methanol (0.7 ml) and H₂O (0.7 ml) at 40 25°C was added KOH (14.3 mg, 0.25 mmole). Consequently, the mixture was heated to 70°C for 2 hours. The reaction was then cooled down to 0°C, diluted with 10 ml of diethyl ether), and acidified with HCl (0.12M in HCl, 2.17 ml). Aqueous workup in the usual manner (H₂O, brine, MgSO₄) gave 45 a mixture of 9-cis and 9,13-dicis acid. Flash column chromatography (silica, 13% ethyl acetate in benzene) gave pure 9-phenyl-9-cis retinoic acid (14.5 mg, 100%).

The ^1H NMR spectrum of 9-phenyl-9-cis retinoic acid is as follows:

5 ^1H NMR (400 MHz), CDCl_3) δ 7.4 - 7.3 (m, 5H, aromatic), 7.20 (dd, J = 16, 12 Hz, 1H, olefinic), 6.60 (d, J = 16 Hz, 1 H, olefinic), 6.38 (d, J = 16 Hz, 1 H, olefinic), 6.25 (d, J = 12 Hz, 1H, olefinic), 6.15 (d, J = 16 Hz, 1 H, olefinic), 5.80 (s, 1H, olefinic), 2.48 (s, 3H, CH_3 l, 2.05 (t, J = 5Hz, 10 2H, CH_2), 1.79 (s, 3H, CH_3), 1.70 - 1.40 (m, 4H, CH_2-CH_2), 1.00 (s, 6H, 2 x CH_3).

9-phenyl-9-cis RA : TLC Rf 0.23 (13% ethyl acetate in Benzene)

15

Example 11

Synthesis of 4-hydroxy-9-cis-retinoic acid

To a solution of 9-cis-retinoic acid (51 mg, 0.17 mmole) in 1,4-dioxane (2 ml) was added SeO_2 (19 mg, 0.17 mmole) at 60°C. The solution was allowed to stir at that temperature for 3 hours. The reaction mixture was then filtered through a silica bed. The filtrate was concentrated and the residue subjected to flash column chromatography (silica, 75% ether in petroleum ether) to afford 4-OH-9-cis-retinoic acid (21 mg., 40% yield), which is characterized as follows: Oil; TLC Rf = 0.25 (silica, 75% ether in petroleum ether); ^1H NMR (400 MHz, CDCl_3) δ 7.08 (dd, J = 16, 12 Hz, 1H, olefinic); 6.64 (d, J = 16 Hz, 1H, olefinic), 6.21 (d, J = 16 Hz, 1H, olefinic), 6.20 (d, J = 16 Hz, 1H, olefinic); 6.04 (d, J = 12 Hz, olefinic), 5.79 (s, 1H, olefinic), 4.02 (t, J = 5 Hz, 1H, $\text{CH}-\text{O}$), 2.18 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.82 (s, 3H, CH_3), 2.0-1.6 (m, 4H, CH_2-CH_2), 1.05, 1.03 (2 x s, 2 x 3H, 2 x CH_3).

Example 12Synthesis of 4-keto-9-cis-retinoic acid

To a solution of 4-hydroxy-9-cis-retinoic acid
5 (16 mg, 0.05 mmole) in CH₂Cl₂ (1.5 ml) was added Dess-Martin
reagent [see Dess and Martin in J. Org. Chem. 48:4155
(1983)] (42 mg, 0.1 mmole) in one portion at 25°C. After
stirring for 5 minutes, the mixture was diluted with 10 ml
of ether and to this was added saturated aqueous NaHCO₃ (5
10 ml) containing Na₂SO₃ (55 mg). The mixture was stirred for
20 minutes to dissolve the solid and the layers separated.
The ether layer was washed with H₂O (2 x 5 ml), brine (5 ml)
and dried (MgSO₄). The solvent was recovered under reduced
pressure and residue was subjected to flash column
15 chromatography (silica, 60% ether in Hexane) to give 4-
keto-9-cis-retinoic acid (14 mg, 90%), characterized as
follows: TLC rf = 0.6 (silica, 80% ether in hexane); ¹HNMR
(400 MHz, CDCl₃) δ 7.05 (dd, J = 16, 12 Hz, 1H, olefinic),
6.82 (d, J = 16 Hz, 1 H, olefinic), 6.32 (d, J = 16 Hz, 1H,
20 olefinic), 6.30 (d, J = 16 Hz, 1H, olefinic), 6.20 (d, J =
12 Hz, 1H, olefinic), 5.80 (s, 1H, olefinic), 2.5 (t, J =
7 Hz, 2H, CH₂-CO), 2.31 (s, 3H, CH₃), 2.01 (s, 3H, CH₃), 1.9
(s, 3H, CH₃), 1.89 (m, 2H, CH₂), 1.20 (s, 6H, 2 x CH₃).

Example 13In vitro evaluation of 9-phenyl-9-cis-retinoic acid,
4-hydroxy-9-cis-retinoic acid and
4-keto-9-cis-retinoic acid

30 The potency and efficacy of the compounds
described in Examples 10, 11 and 12 were determined (as
described in Example 1--under the heading "Cotransfection
Assay in CV-1 Cells". The results are presented in Table
4:

Table 4

	9-cis-retinoic acid	9-phenyl-9-cis-retinoic acid	4-hydroxy-9-cis-retinoic acid	4-keto-9-cis-retinoic acid				
Receptor	Potency (nM)	Efficacy	Potency (nM)	Efficacy	Potency (nM)	Efficacy	Potency (nM)	Efficacy
RXR α	88	170%	210	76%	1700	161%	520	104%
RXR β	61	106%	44	88%	650	143%	1300	105%
RXR γ	360	137%	290	77%	1700	115%	1100	133%
RAR α	99	94%	>10,000	<2%	380	65%	200	50%
RAR β	22	97%	880	39%	160	71%	26	67%
RAR γ	43	108%	250	59%	180	81%	55	107%

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and
5 claimed.

SEQUENCE LISTING

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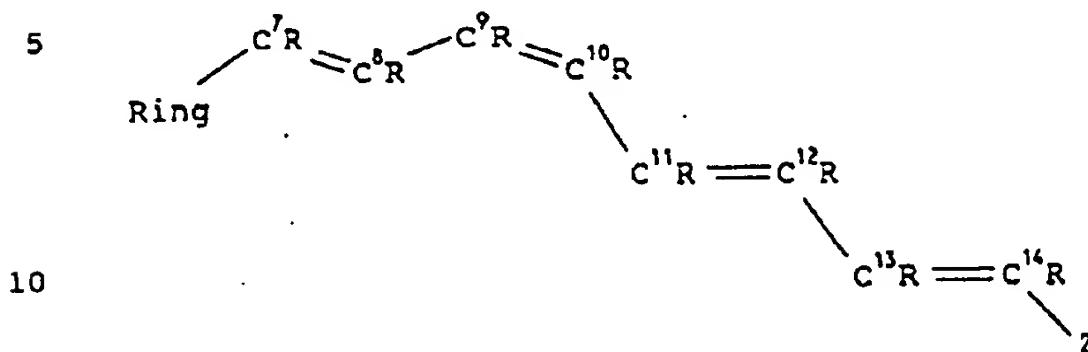
Cys - X - X - Cys - X - X - Asp* - X - Ala* - X -
Gly* - X - Tyr* - X - X - X - X - Cys - X - X - Cys -
Lys* - X - Phe - Phe - X - Arg* - X - X - X - X - X -
X - X - X - (X - X -) Cys - X - X - X - X - X - (X -
X - X -) Cys - X - X - Lys - X - X - Arg - X - X -
Cys - X - X - Cys - Arg* - X - X - Lys* - Cys - X - X -
X - Gly* - Met

SEQ ID NO:2

5' -TCAGCTCATGACCTGA-3'

That which is claimed is:

1. A method for modulating process(es) mediated by retinoid receptors, said method comprising conducting said process(es) in the presence of at least one compound of the structure:



wherein:

unsaturation between carbon atoms C⁹ and C¹⁰ has a cis configuration, and one or both sites of unsaturation between carbon atoms C¹¹ through C¹⁴ optionally have a cis configuration;

"Ring" is a cyclic moiety;

Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, or carbamate; and

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents; or

any two or more of the R groups can be linked to one another to form one or more ring structures.

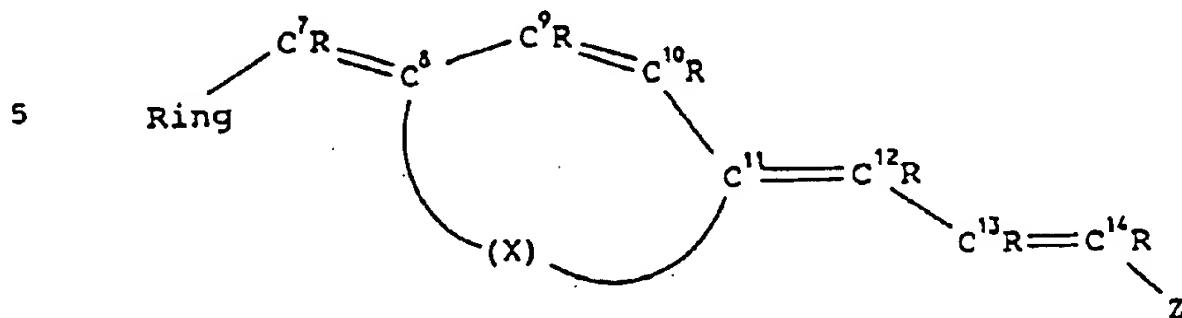
2. A method according to claim 1 wherein said retinoid receptor is selected from retinoic acid receptor-alpha, retinoic acid receptor-beta, or retinoic acid receptor-gamma.

3. A method according to claim 1 wherein said retinoid receptor is selected from retinoid X receptor-alpha, retinoid X receptor-beta, or retinoid X receptor-gamma.

4. A method according to claim 1 wherein said process is selected from *in vitro* cellular differentiation, *in vitro* cellular proliferation, *in vitro* proliferation of melanoma cell lines, *in vitro* 5 differentiation of mouse teratocarcinoma cells (F9 cells), *in vitro* differentiation of human epidermal keratinocytes, regulation of cellular retinol binding protein (CRBP), or *in vitro* limb morphogenesis.

5. A method according to claim 1 wherein said process is selected from the *in vivo* modulation of lipid metabolism, *in vivo* modulation of skin-related processes, or *in vivo* modulation of malignant cell 5 development.

6. A method according to claim 1 wherein said compound has the structure (I): .



10 Structure I

wherein:

x is $-[(CR_2)_x - x' - (CR_2)_y] -$

X' is selected from -O-, carbonyl, -S-,

-S(O)-, -S(O)₂-, thiocarbonyl, -NR^{II}-, or -CR₂-.

"Ring" is a cyclic moiety;

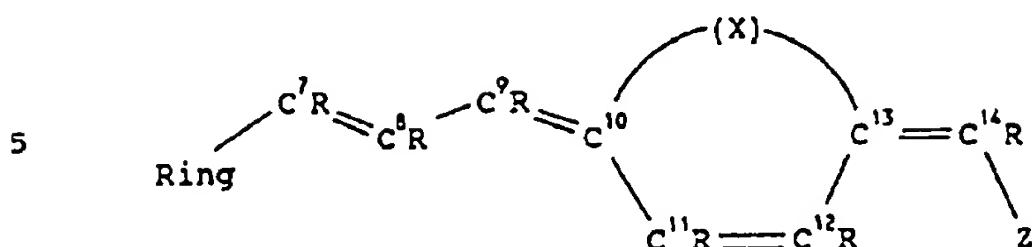
Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, or carbamate; and

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, or amino;

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl;

x is 0, 1 or 2,
y is 0, 1, or 2, and
x + y ≤ 2.

7. A method according to claim 1 wherein said compound has the structure (II):



10

Structure II

wherein:

X is $-((CR_2)_x - X' - (CR_2)_y) -$,

X' is selected from -O-, carbonyl, -S-,

-S(O)-, -S(O)₂-, thiocarbonyl, -NRⁿ-, or -CR₂-,

"Ring" is a cyclic moiety;

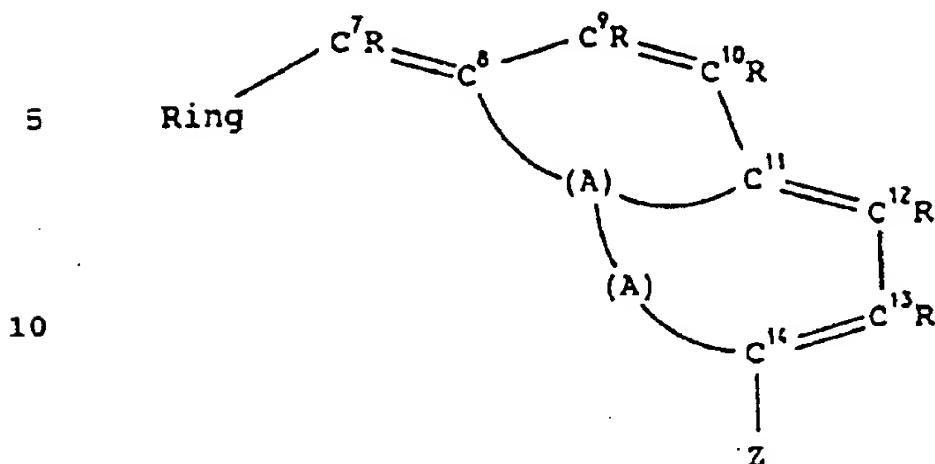
15 "Ring" is a cyclic moiety;
20 Z is selected from carboxyl, carboxaldehyde,
hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate,
alkyl ether of a hydroxyalkyl group, alkyl
thioether of a thioalkyl group, esters of
hydroxyalkyl groups, thioesters of hydroxyalkyl
group, esters of thioalkyl groups, thioesters of
thioalkyl groups, aminoalkyl, N-acyl aminoalkyl,
or carbamate; and

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

Rⁿ is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl;

30 x is 0, 1 or 2,
 y is 0, 1, or 2, and
 x + y ≤ 2.

8. A method according to claim 1 wherein said compound has the structure (III):

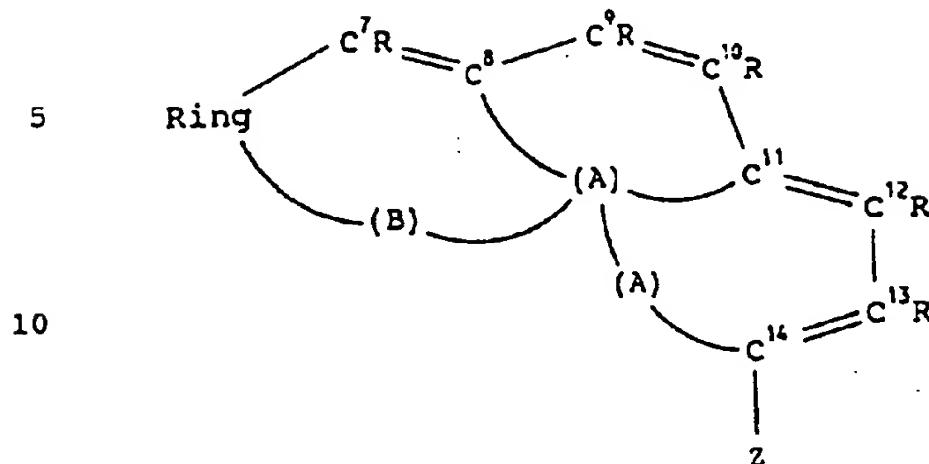


Structure III

wherein:

- 15 one A is X and the other A is X',
X is $-(CR_2)_x-X'-(CR_2)_y-$,
X' is selected from -O-, carbonyl, -S-,
-S(O)-, -S(O)₂-, thiocarbonyl, -NR"-, or -CR₂-,
"Ring" is a cyclic moiety;
- 20 Z is selected from carboxyl, carboxaldehyde,
hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate,
alkyl ether of a hydroxyalkyl group, alkyl
thioether of a thioalkyl group, esters of
hydroxyalkyl groups, thioesters of hydroxyalkyl
group, esters of thioalkyl groups, thioesters of
thioalkyl groups, aminoalkyl, N-acyl aminoalkyl,
or carbamate; and
- 25 each R is independently selected from H,
halogen, alkyl, aryl, hydroxy, thiol, alkoxy,
thioalkoxy, amino, or any of the Z substituents;
- 30 R" is hydrogen, alkyl, hydroxy, thiol, or
alkoxy acyl;
x is 0, 1 or 2,
y is 0, 1, or 2, and
x + y ≤ 2.
- 35

9. A method according to claim 1 wherein said compound has the structure (IV):

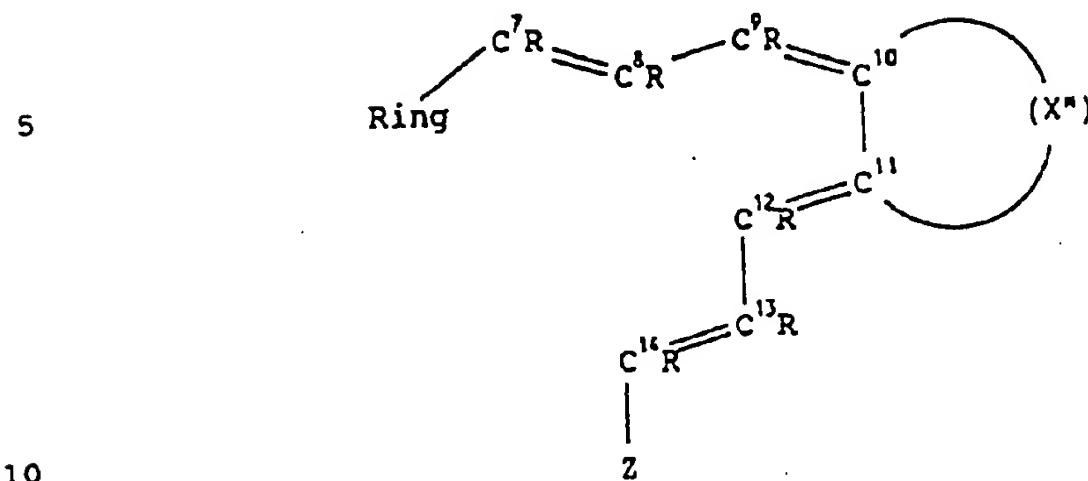


Structure IV

wherein:

- 15 one A is X and the other A is X',
 B is X',
 X is $-[(CR_2)_x-X'-(CR_2)_y]-$,
 X' is selected from -O-, carbonyl, -S-,
 $-S(O)-$, $-S(O)_2-$, thiocarbonyl, $-NR''-$, or $-CR_2-$,
- 20 "Ring" is a cyclic moiety;
 Z is selected from carboxyl, carboxaldehyde,
 hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate,
 alkyl ether of a hydroxyalkyl group, alkyl
 thioether of a thioalkyl group, esters of
 hydroxyalkyl groups, thioesters of hydroxyalkyl
 group, esters of thioalkyl groups, thioesters of
 thioalkyl groups, aminoalkyl, N-acyl aminoalkyl,
 or carbamate; and
- 25 each R is independently selected from H,
 halogen, alkyl, aryl, hydroxy, thiol, alkoxy,
 thioalkoxy, amino, or any of the Z substituents;
- 30 R'' is hydrogen, alkyl, hydroxy, thiol, or
 alkoxy acyl;
- 35 x is 0, 1 or 2,
 y is 0, 1, or 2, and
 x + y ≤ 2.

10. A method according to claim 1 wherein said compound has the structure (V):



Structure V

wherein:

X'' is $-[(CR_2)_a - X' - (CR_2)_b] -$,

x' is selected from -O-, carbonyl, -S-,

-S(O)-, -S(O)₂-, thiocarbonyl, -NR²⁺-, or -CR₂-.

"Ring" is a cyclic moiety;

Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, or carbamate; and

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

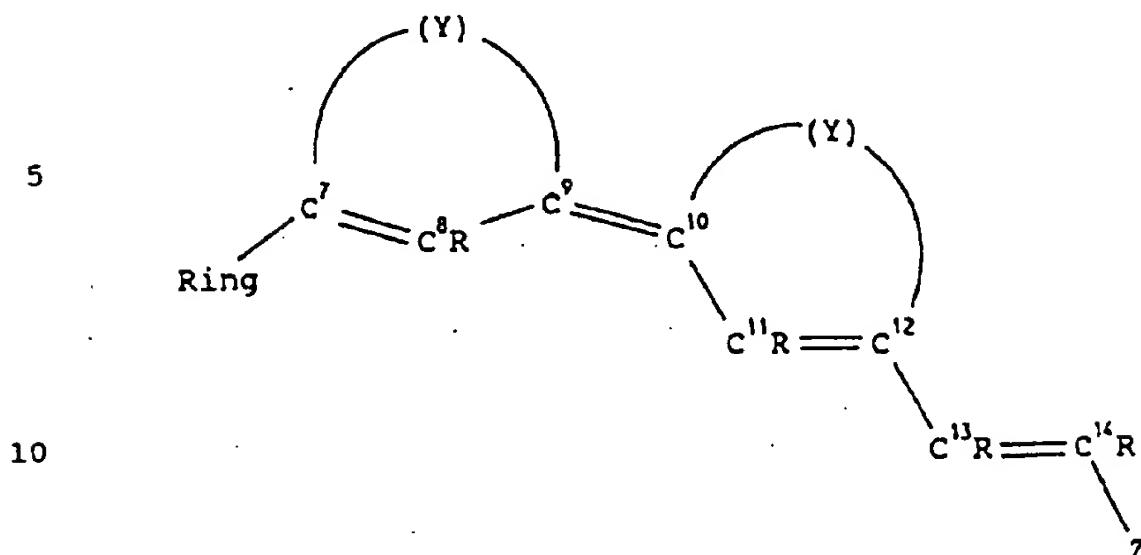
R" is hydrogen, halogen, alkyl, hydroxy, or thiol;

30 a is 0, 1, 2, 3 or 4.

b is 0, 1, 2, 3, or 4, and

a + b is ≥ 2 , but ≤ 4 .

11. A method according to claim 1 wherein said compound has the structure (VI):

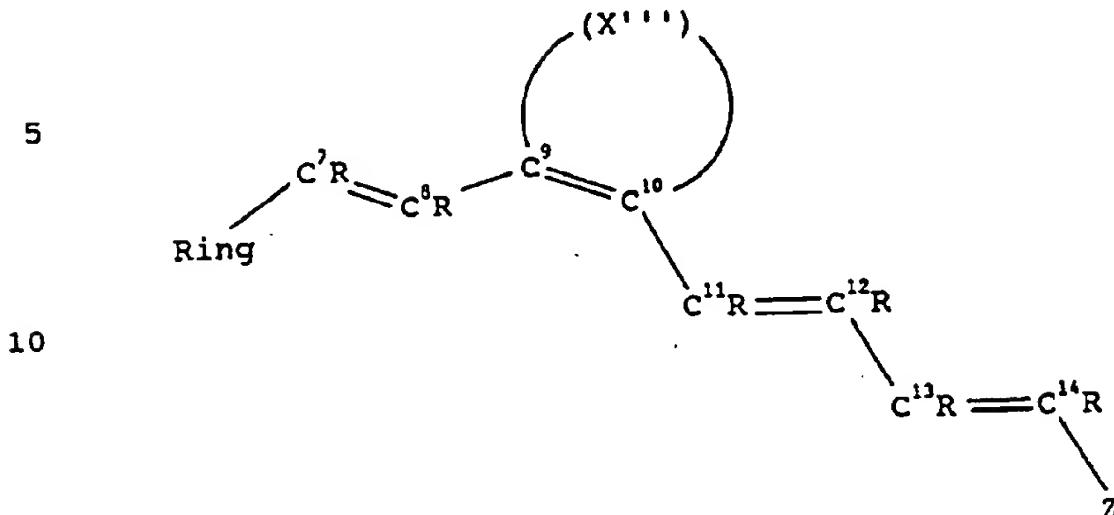


Structure VI

wherein:

- 15 Y is $-[(CR_2)_c-X'-(CR_2)_d]-$,
 X' is selected from -O-, carbonyl, -S-,
 -S(O)-, -S(O)₂-, thiocarbonyl, -NR"-, or -CR₂-,
 "Ring" is a cyclic moiety;
- 20 Z is selected from carboxyl, carboxaldehyde,
 hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate,
 alkyl ether of a hydroxyalkyl group, alkyl
 thioether of a thioalkyl group, esters of
 hydroxyalkyl groups, thioesters of hydroxyalkyl
 group, esters of thioalkyl groups, thioesters of
 thioalkyl groups, aminoalkyl, N-acyl aminoalkyl,
 or carbamate; and
- 25 each R is independently selected from H,
 halogen, alkyl, aryl, hydroxy, thiol, alkoxy,
 thioalkoxy, amino, or any of the Z substituents;
- 30 R" is hydrogen, alkyl, hydroxy, thiol, or
 alkoxy acyl;
 c is 0, 1, 2 or 3,
 d is 0, 1, 2 or 3, and
 c + d ≥ 1, but ≤ 3.

12. A method according to claim 1 wherein said compound has the structure (VII):



Structure VII

wherein;

15 X''' is X" or an unsaturated linking group having the structure:

-[Q = CR - J]-,

wherein Q is -N= or -CR=, and J is -CR=CR-,
 -N=CR-, -CR=N-, -O-, -S-, or -NR"-,

20 thereby incorporating C⁹ and C¹⁰ of the rexoid compound into an aromatic (or pseudo-aromatic) ring,

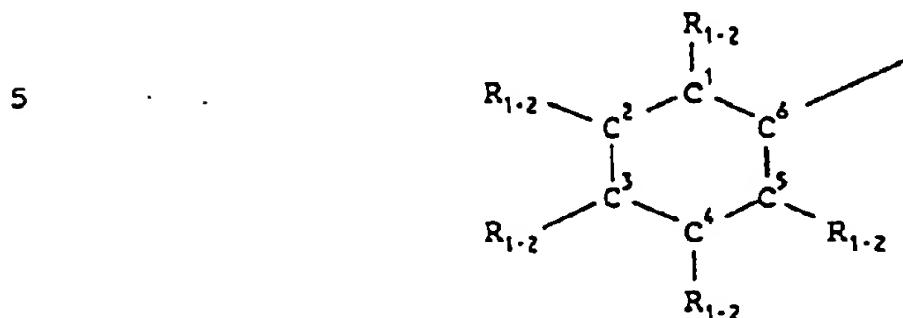
X" is $-[(CR_2)_a-X'-(CR_2)_b]-$,
 X' is selected from -O-, carbonyl, -S-,
 -S(O)-, -S(O)₂-, thiocarbonyl, -NR"-, or -CR₂-,
 "Ring" is a cyclic moiety;

30 z is selected from carboxyl, carboxaldehyde,
hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate,
alkyl ether of a hydroxyalkyl group, alkyl
thioether of a thioalkyl group, esters of
hydroxyalkyl groups, thioesters of hydroxyalkyl
group, esters of thioalkyl groups, thioesters of
thioalkyl groups, aminoalkyl, N-acyl aminoalkyl,
or carbamate; and

35 each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents; R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl;

40 a is 0, 1, 2, 3 or 4,
b is 0, 1, 2, 3, or 4, and
a + b is ≥ 2 , but ≤ 4 .

13. A method according to claim 1 wherein Ring has the following structure:

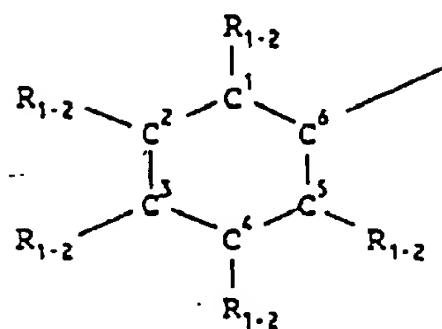


10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;
any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-; R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

14. A method according to claim 6 wherein
Ring has the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents; any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-; thiocarbonyl (>CS), or -NR"-;

15

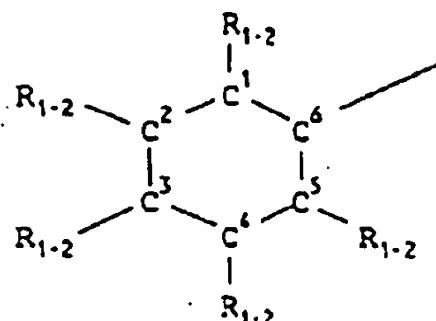
R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

20

said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof, or an aromatic derivative thereof.

15. A method according to claim 7 wherein
Ring has the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

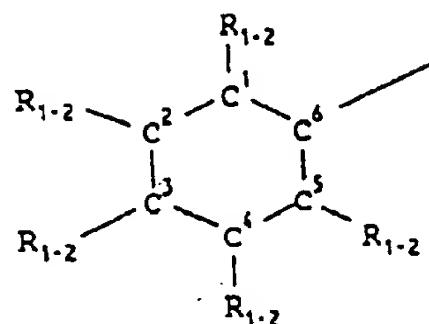
15 any one of C², C³, or C⁵ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

20 said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof.

16. A method according to claim 8 wherein
Ring has the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents; any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

15

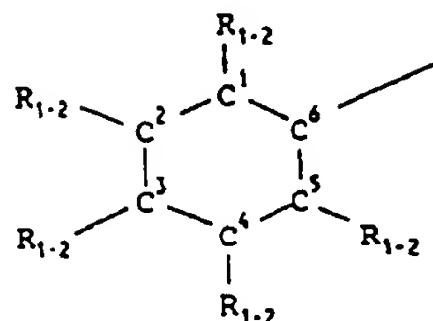
R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

20

said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

17. A method according to claim 9 wherein
Ring has the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

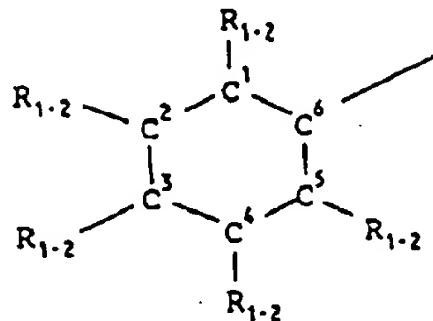
any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

18. A method according to claim 10 wherein
Ring has the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

15

any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

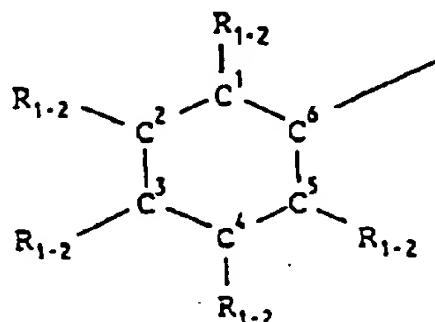
20

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

19. A method according to claim 11 wherein
Ring has the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

15 any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

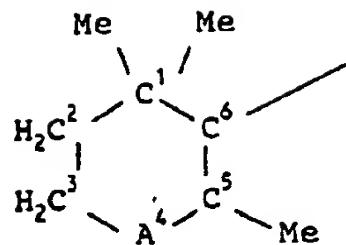
R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

20 said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

20. A method according to claim 1 wherein said compound is selected from 9-cis-retinoic acid, 9-phenyl-9-cis-retinoic acid, 4-hydroxy-9-cis-retinoic acid, 4-keto-9-cis-retinoic acid, 9,11-dicis retinoic acid, 5 and 9-cis-locked derivatives of retinoic acid selected from Structures I-VII as set forth in the specification, wherein Z is carboxyl and Ring is a β -ionone or β -ionone-like species having the structure:

69

10

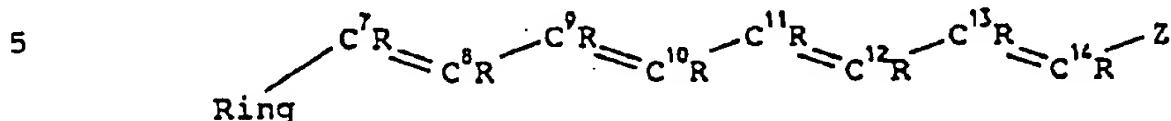


15 wherein A' is selected from >CH₂, >C=O or >C-OH.

21. A method according to claim 1 wherein Ring has four or five carbon atoms and is selected from cyclopentane, cyclopentene, dihydropyran, tetrahydropyran, piperidine, dihydrothiopyran, tetrahydrothiopyran, 5 dihydrofuran, tetrahydrofuran, tetrahydrothiophene, pyrrolidine, or derivatives thereof.

22. A method to modulate processes mediated by retinoid receptors, said method comprising conducting said process in the presence of:

(a) at least one compound of the structure:



wherein:

10 each site of unsaturation in the side chain comprising carbon atoms C⁷ through C¹⁴ has a trans configuration;

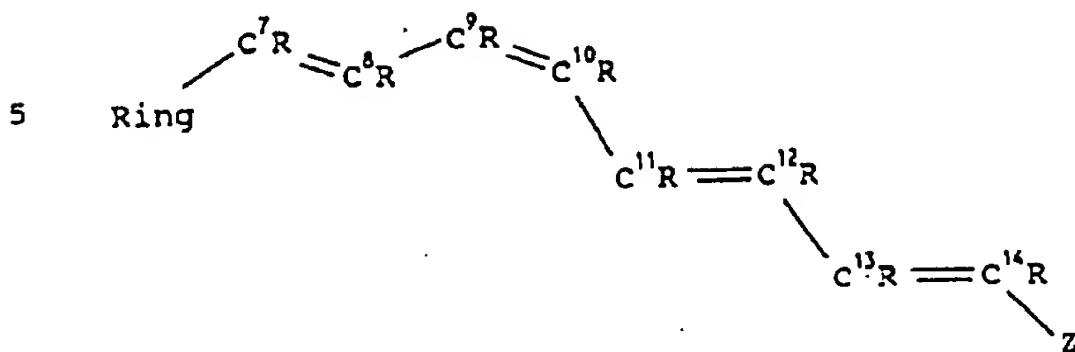
"Ring" is a cyclic moiety;

15 Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, carbamate, and the like; and

20 each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents; and

25 (b) a cis/trans isomerase capable of converting at least one of the 9-, 11-, or 13-double bonds from the trans configuration to the cis-configuration.

23. A method to produce compound(s) of the structure:



10 wherein:

unsaturation between carbon atoms C^9 and C^{10} has a cis configuration, and one or both sites of unsaturation between carbon atoms C^{11} through C^{14} optionally have a cis configuration;

15 "Ring" is a cyclic moiety;

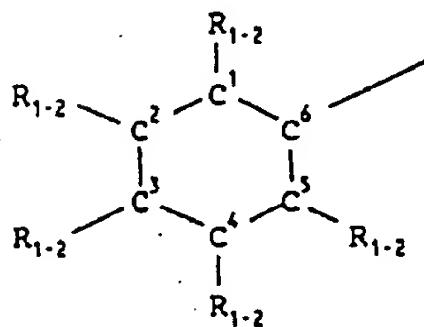
Z is selected from carboxyl, carboxaldehyde, hydroxyalkyl, thioalkyl, hydroxyalkyl phosphate, alkyl ether of a hydroxyalkyl group, alkyl thioether of a thioalkyl group, esters of hydroxyalkyl groups, thioesters of hydroxyalkyl group, esters of thioalkyl groups, thioesters of thioalkyl groups, aminoalkyl, N-acyl aminoalkyl, carbamate, and the like; and

20 25 each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

from the corresponding all-trans configuration material, said method comprising contacting said all-trans configuration material with a cis/trans isomerase under 30 isomerization conditions.

24. A method according to claim 23 wherein
Ring is a cyclohexyl ring having the following structure:

5



10 wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

15 any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

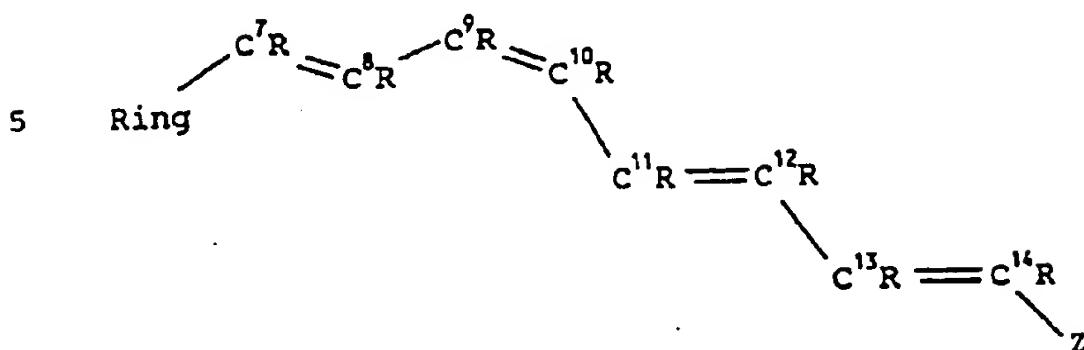
20 said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof.

25. A method according to claim 23 wherein
said contacting is carried out *in vivo*.

26. A method according to claim 25 wherein
said contacting is carried out in Schneider cells.

27. A method according to claim 23 wherein
said contacting is carried out *in vitro*.

28. Composition comprising at least one compound having a structure selected from:



10 Structure A

wherein:

unsaturation between carbon atoms C^9 and C^{10} has a cis configuration, and one or both sites of unsaturation between carbon atoms C^{11} through C^{14} optionally have a cis configuration;

15 "Ring" is a cyclic moiety, optionally having one or more substituents thereon;

20 Z is selected from carboxyl (-COOH), carboxaldehyde (-COH), hydroxyalkyl $[-(CR')_n-OH]$, wherein each R' is independently selected from hydrogen or a lower alkyl and n falls in the range of 1 up to about 4], thioalkyl $[-(CR')_n-SH]$, wherein R' and n are as defined above], hydroxyalkyl phosphate $[-(CR')_n-OP(OM)_3]$, wherein R' and n are as defined above and M is hydrogen, lower alkyl, or a cationic species such as Na⁺, Li⁺, K⁺, and the like], alkyl ether of a hydroxyalkyl group $[-(CR')_n-OR']$, wherein R' and n are as defined above], alkyl thioether of a thioalkyl group $[-(CR')_n-SR']$, wherein R' and n are as defined above], esters of hydroxyalkyl groups $[-(CR')_n-O-CO-R']$, wherein R' and n are as defined above], thioesters of hydroxyalkyl group $[-(CR')_n-O-CS-R']$, wherein R' and n are as defined above], esters of thioalkyl groups $[-(CR')_n-S-CO-R']$, wherein R' and n are as defined above]

25

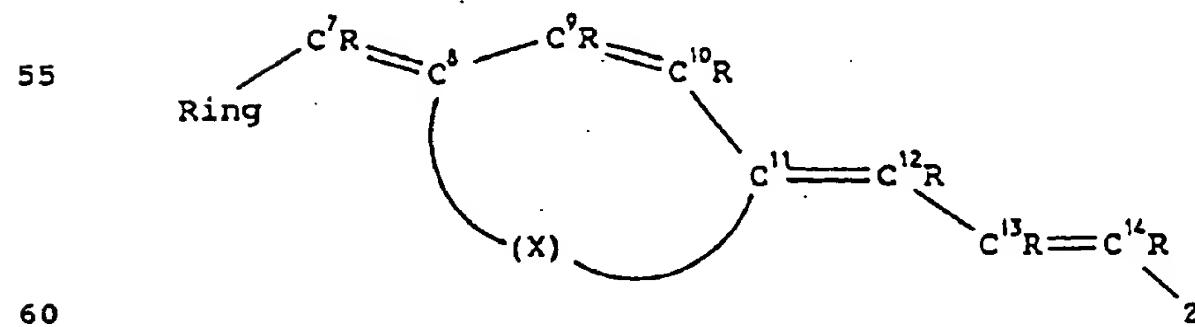
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35

above], thioesters of thioalkyl groups $[-(CR')_n-S-CS-R']$, wherein R' and n are as defined above], aminoalkyl $[-(CR')_n-NR'^2]$, wherein R' and n are as defined above], N-acyl aminoalkyl $[-(CR')_n-NR'-CO-R'']$, wherein R' and n are as defined above and R'' is a lower alkyl or benzyl], carbamate $[-(CR')_n-NR'-CO-OR']$ or $-(CR')_n-O-CO-NR'^2$, wherein R' and n are as defined above]; and

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents, with the proviso that Structure A is not 9-cis-retinoic acid; or

any two or more of the R groups can be linked to one another to form one or more ring structures;



Structure I;

wherein:

"Ring", Z and R are as defined above;

X is $-[(CR_2)_x-X'-(CR_2)_y]-$,

X' is selected from -O-, carbonyl, -S-, $-S(O)-$, $-S(O)_2-$, thiocarbonyl, $-NR''-$, or $-CR_2-$,

R'' is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl;

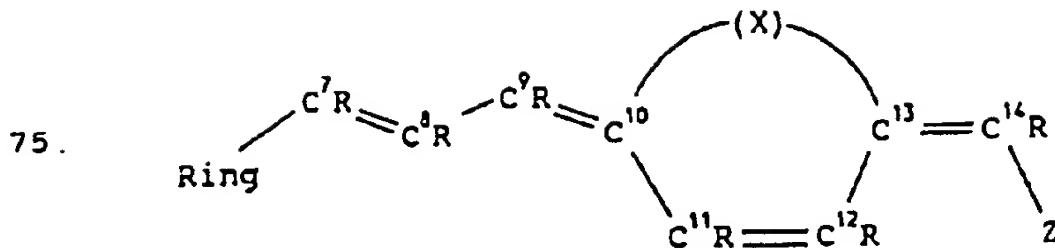
x is 0, 1 or 2,

y is 0, 1, or 2, and

x + y ≤ 2;

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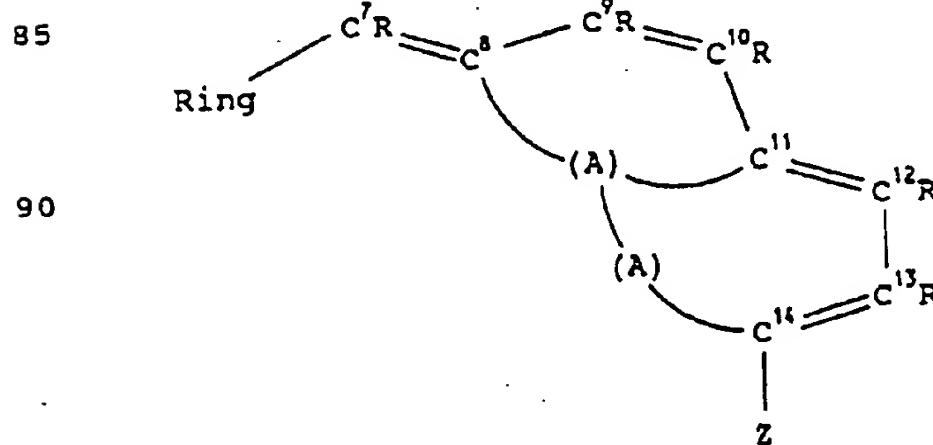


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Structure II;

wherein:

X, X', R, R'', Z, Ring, x and y are as defined above;



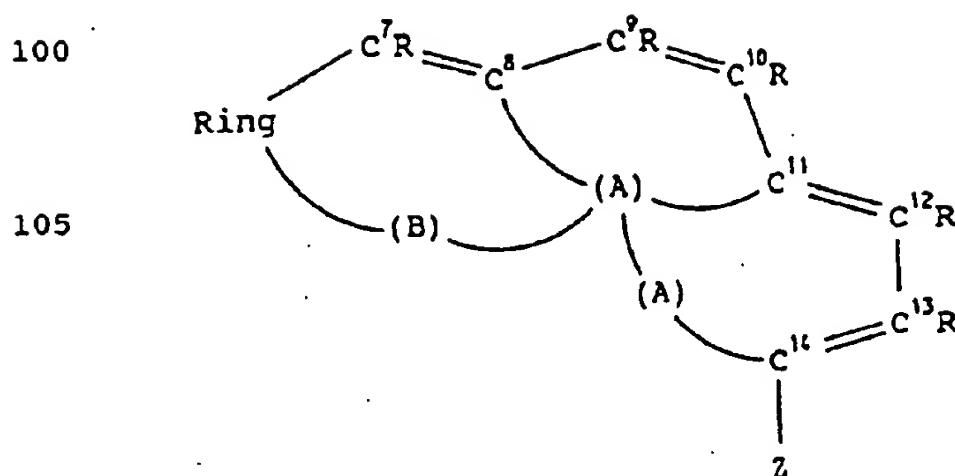
95

Structure III

wherein:

one A is X and the other A is X', and
 X, X', R, R'', Z, Ring, x and y are as defined above;

76



110

Structure IV;

wherein:

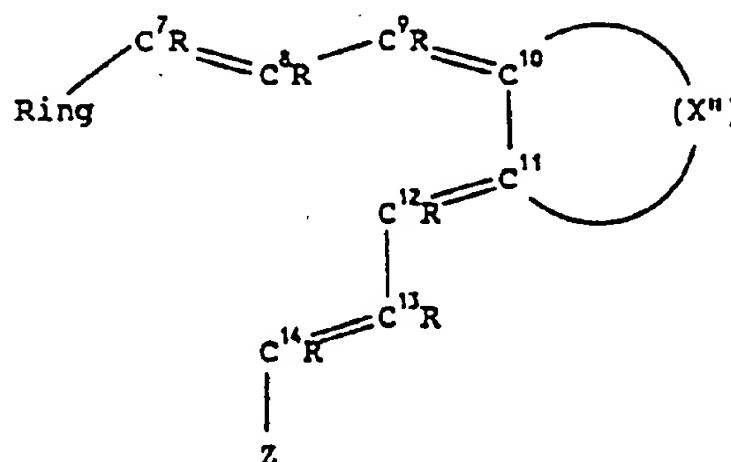
one A is X and the other A is X',

B is X', and

X, X', R, R'', Z, Ring, x and y are as

115 defined above;

120

Structure V;

125 wherein:

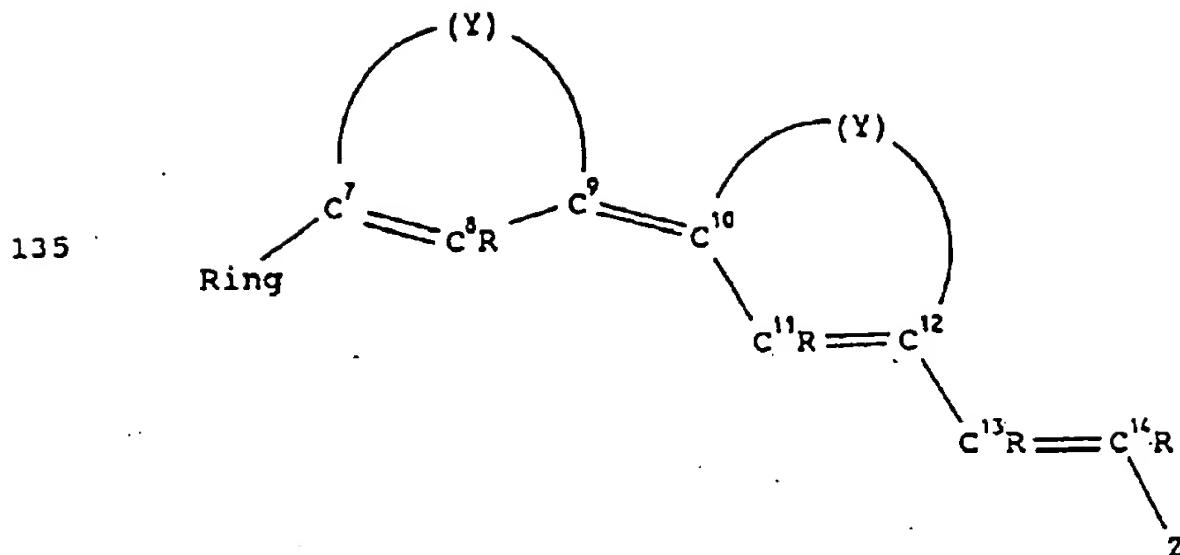
X'' is $-[(\text{CR}_2)_a - \text{X}' - (\text{CR}_2)_b] -$,

X', R, R'', Ring and Z are as defined above,

a is 0, 1, 2, 3 or 4,

b is 0, 1, 2, 3, or 4, and

130 a + b is ≥ 2 , but ≤ 4 ;



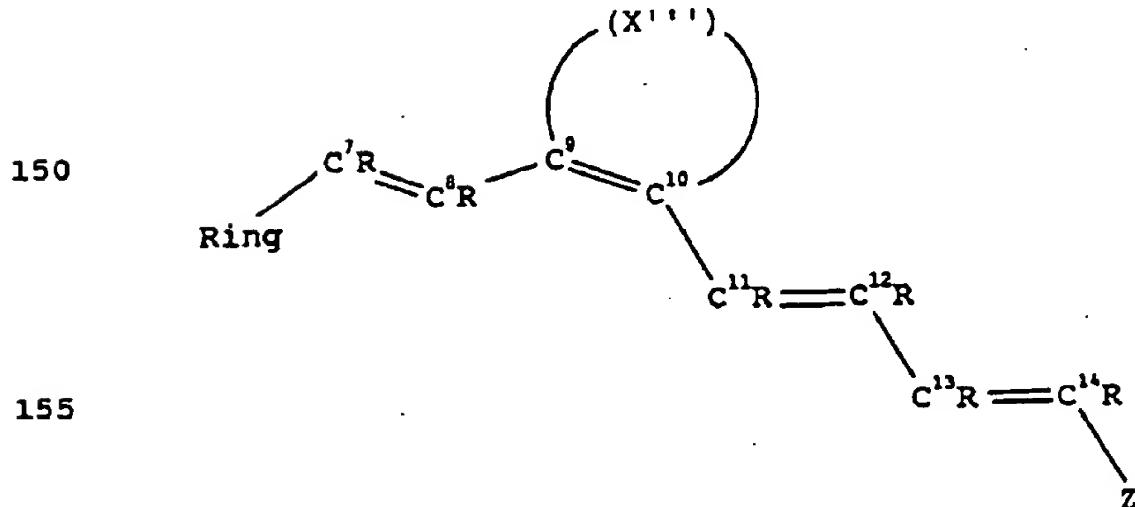
140

Structure VI;

wherein:

145

Y is $-[(CR_2)_c-X'-(CR_2)_d]-$,
 X' , R , R'' , Ring and Z are as defined above,
 c is 0, 1, 2 or 3,
 d is 0, 1, 2 or 3, and
 $c + d \geq 1$, but ≤ 3 ; and

Structure VII

wherein:

160

X''' is X'' or an unsaturated linking group having the structure:

-[Q = CR - J]-,

wherein Q is -N= or -CR=, and J is -CR=CR-,
 -N=CR-, -CR=N-, -O-, -S-, or -NR"-,

165

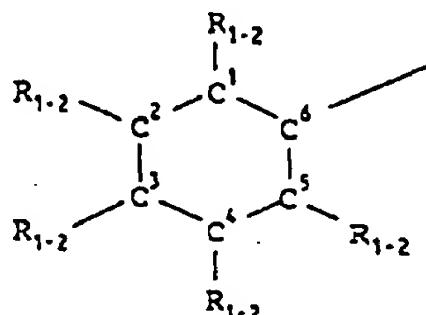
thereby incorporating C⁹ and C¹⁰ of the rexoid compound into an aromatic (or pseudo-aromatic) ring, and

X', X", R, R", Ring, Z, a and b are as defined above.

29. A composition according to claim 28 wherein Ring is a cyclohexyl ring having the following structure:

5

10



wherein:

each R is independently selected from H, halogen, alkyl, aryl, hydroxy, thiol, alkoxy, thioalkoxy, amino, or any of the Z substituents;

15

any one of C², C³, or C⁴ can be replaced with -O-, carbonyl (>CO), -S-, -S(O)-, -S(O)₂-, thiocarbonyl (>CS), or -NR"-;

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy acyl; and

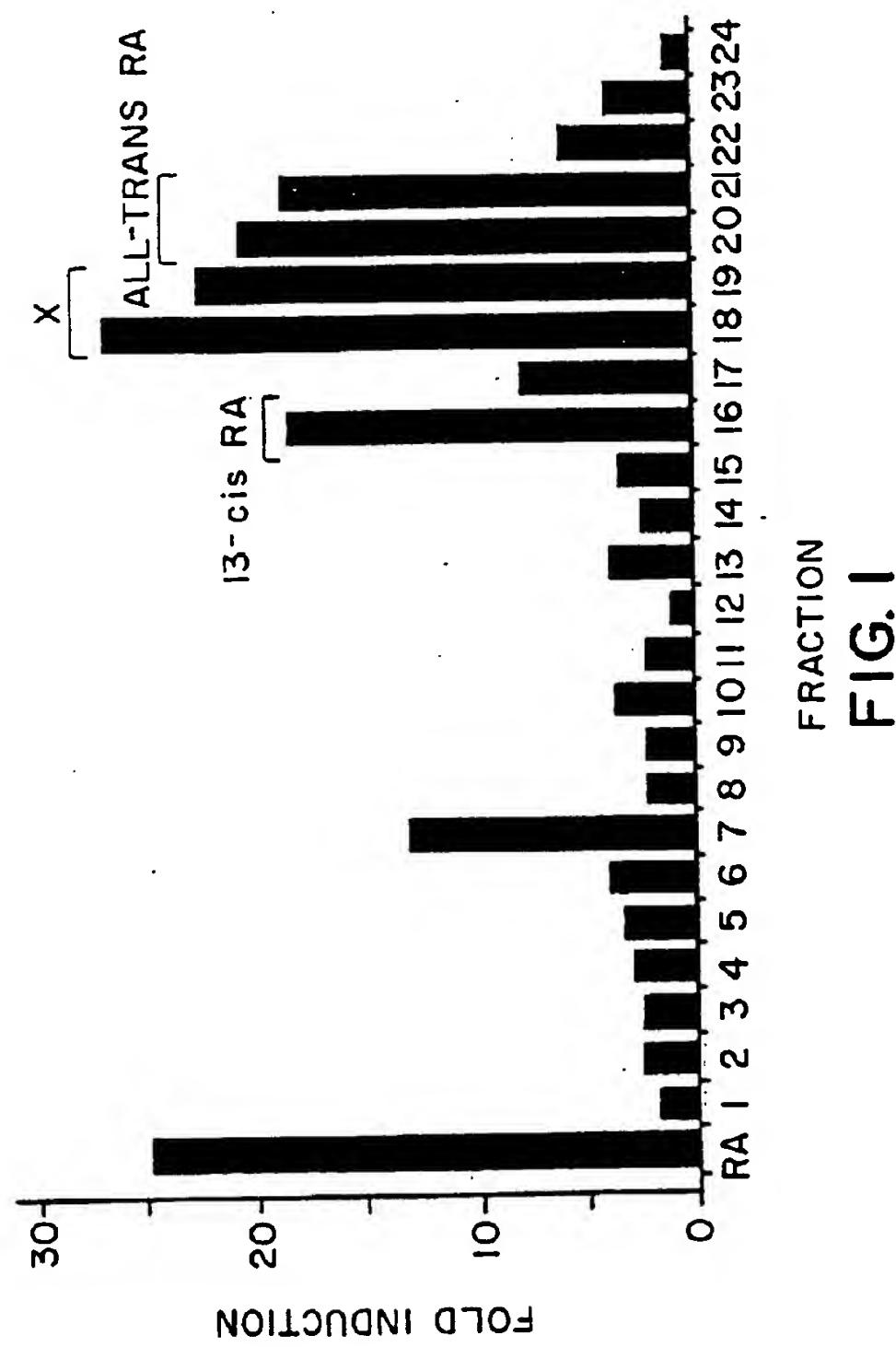
20

said cyclic moiety exists as the saturated, 2-ene, 3-ene, 4-ene, or 5-ene mono-unsaturated isomer, or the 2,4-, 2,5-, or 3,5-diene derivative thereof; or an aromatic derivative thereof.

WO 93/11755

PCT/US92/11214

1/6



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WO 93/11755

PCT/US92/11214

2/6

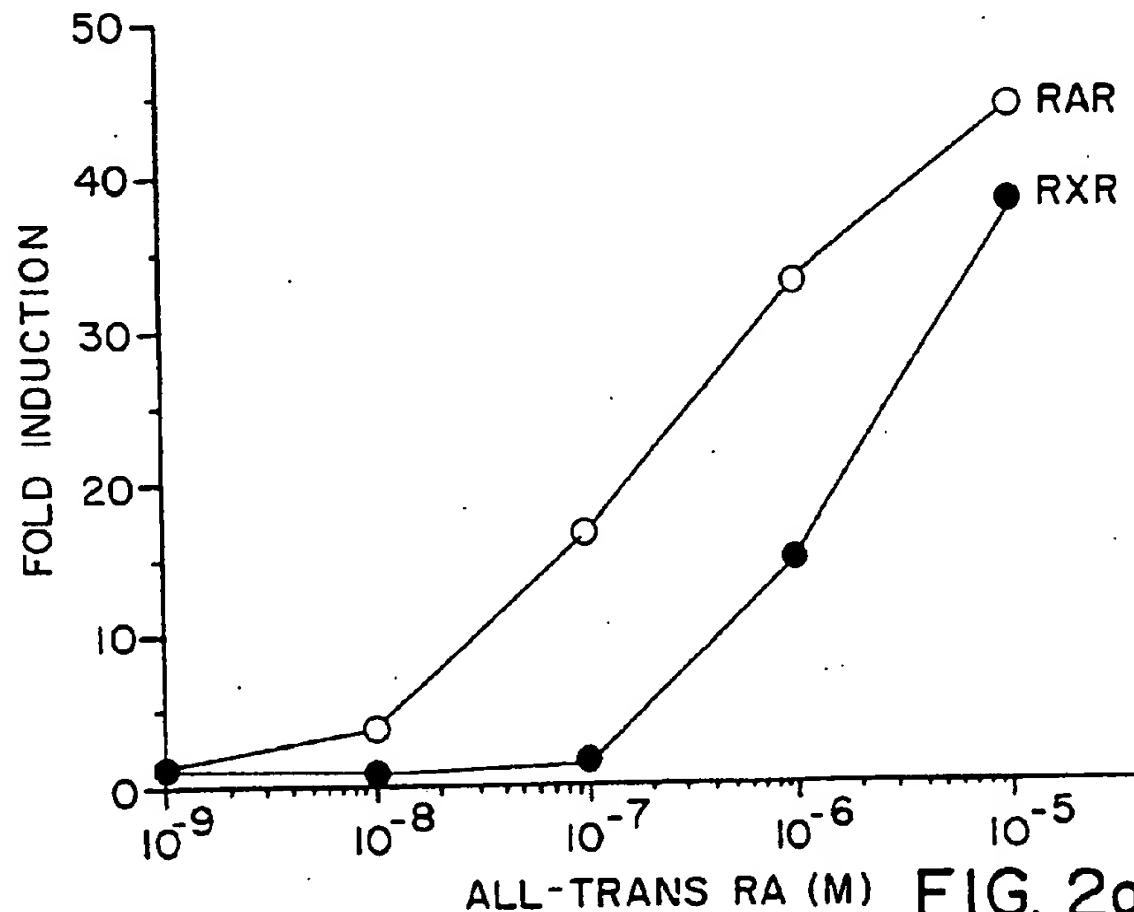


FIG. 2a

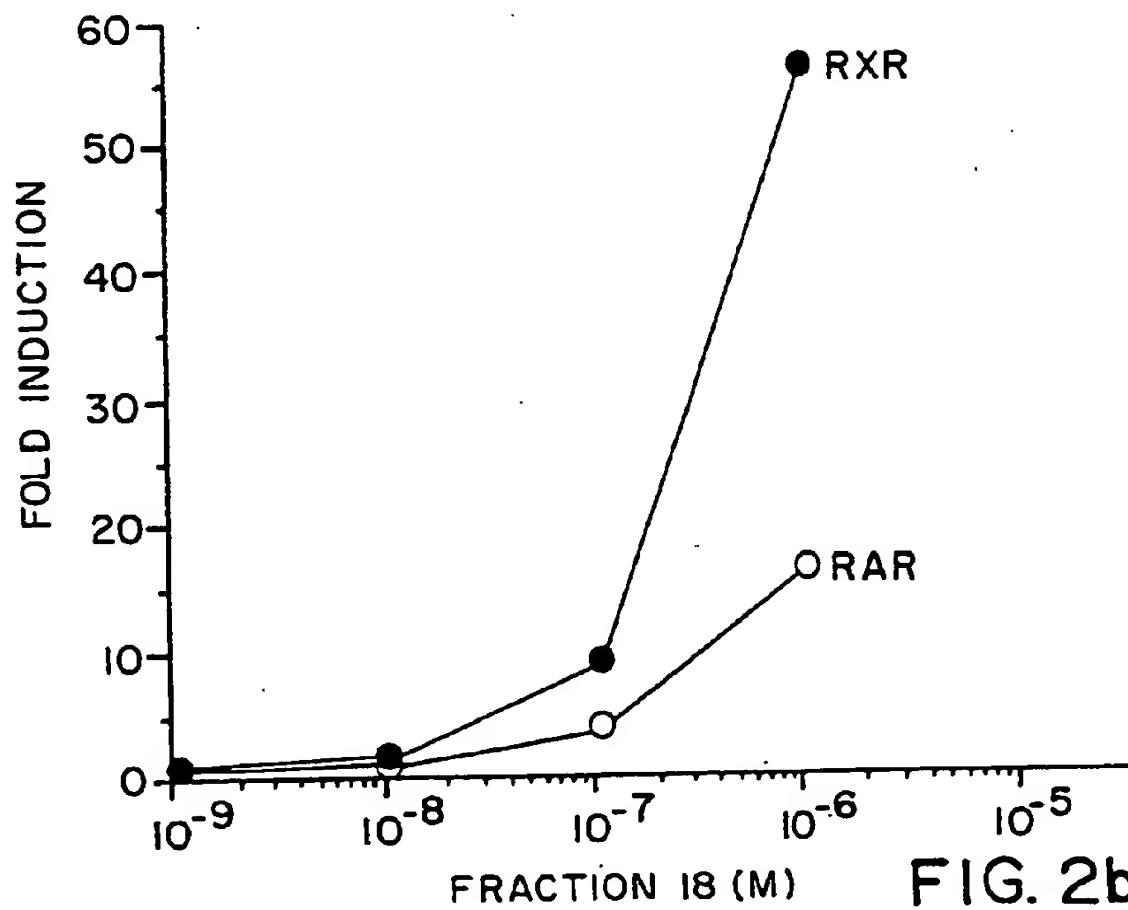


FIG. 2b

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3/6

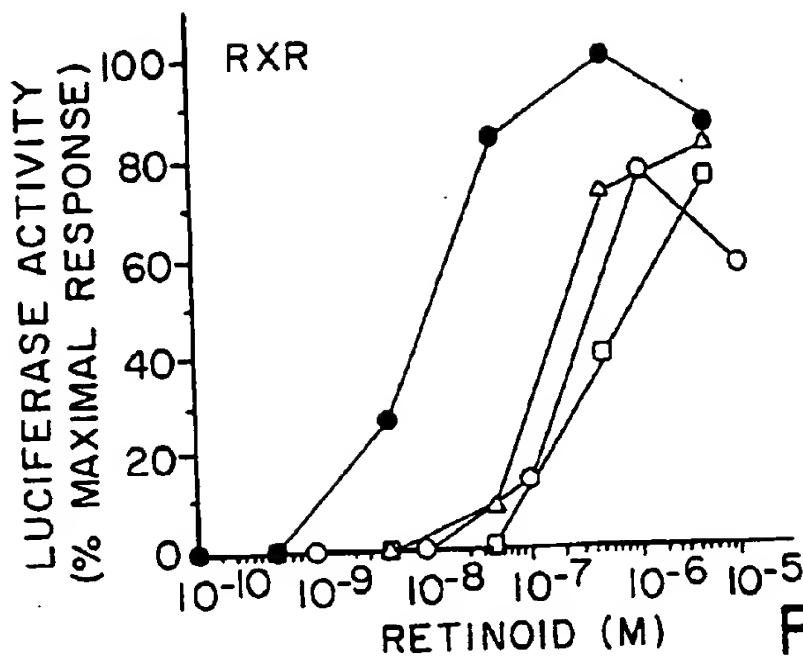


FIG. 3a

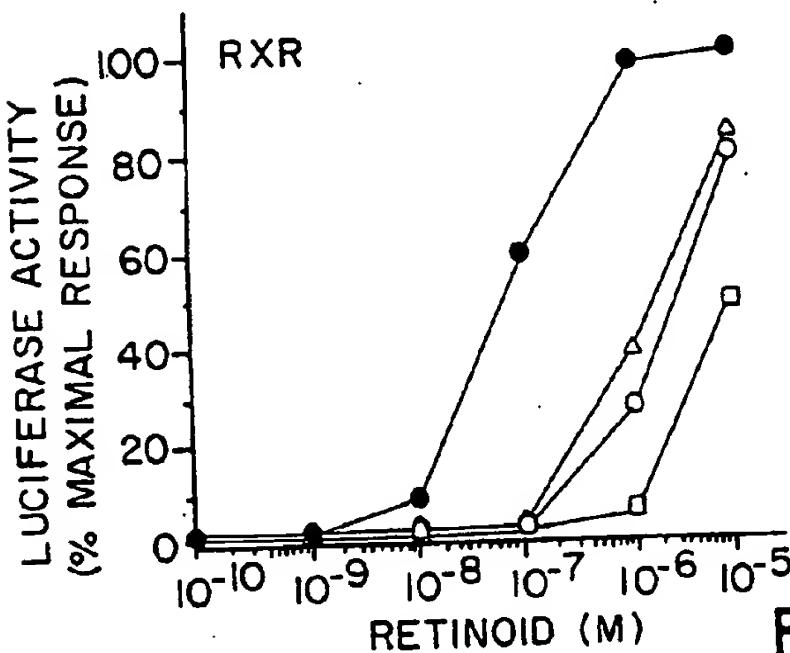


FIG. 3b

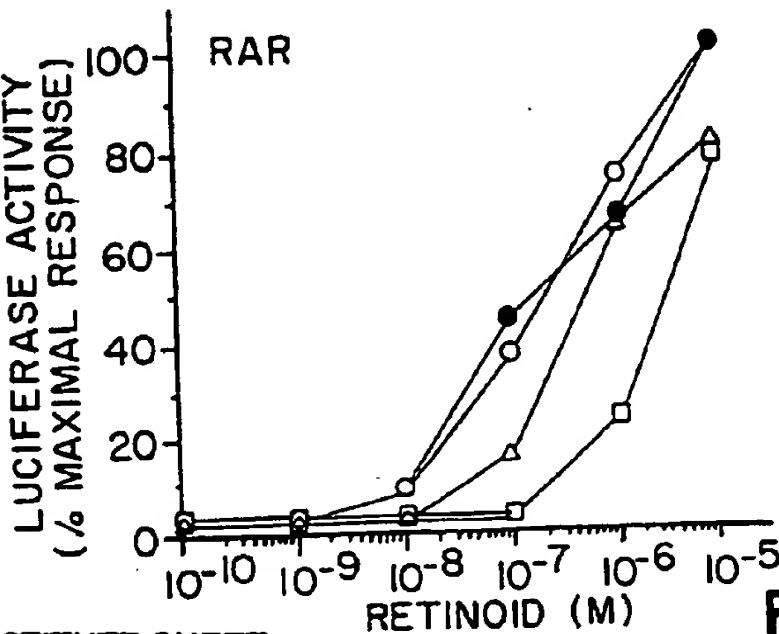


FIG. 3c

4/6

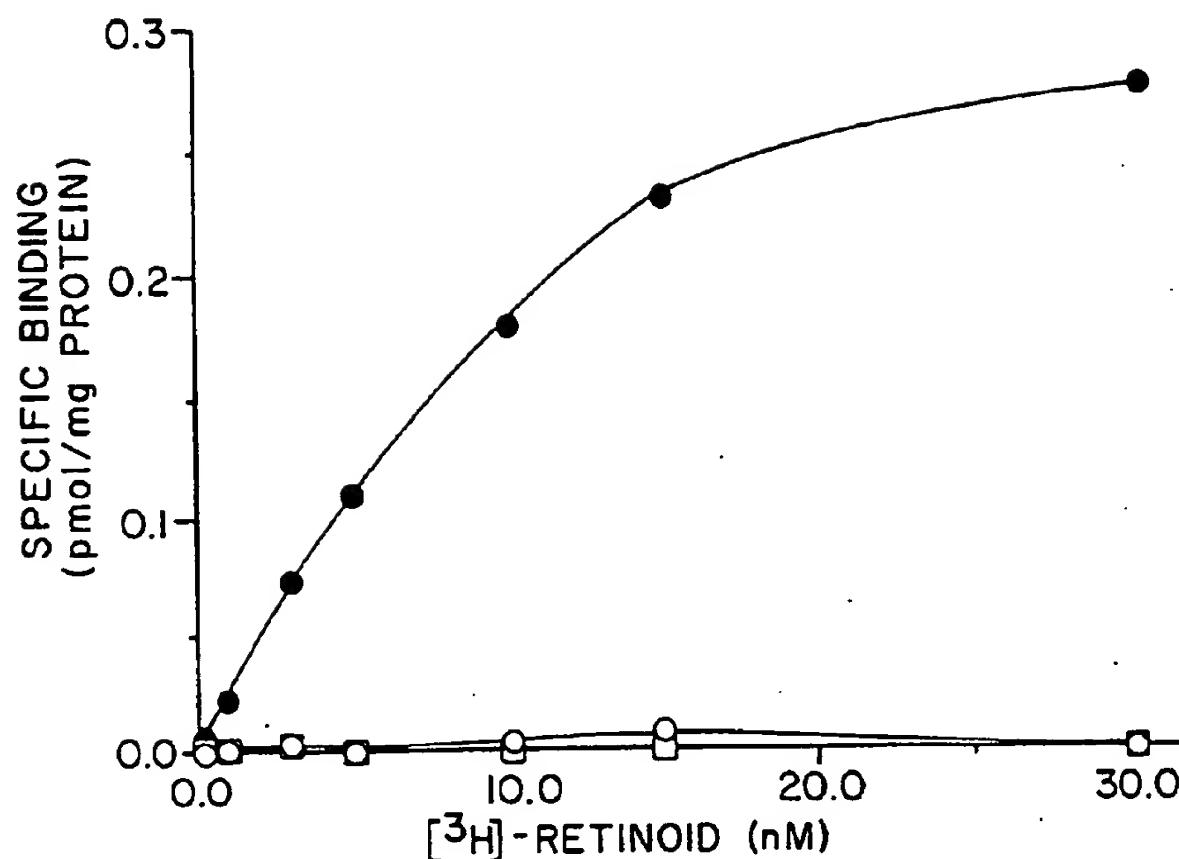


FIG. 4a

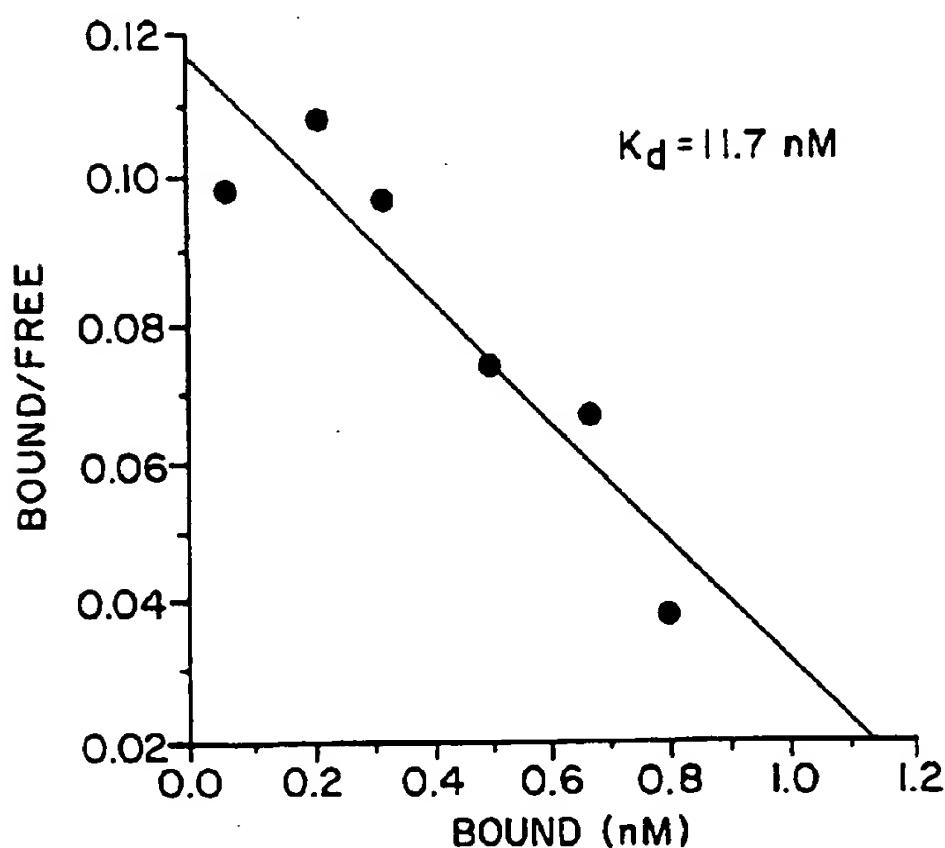
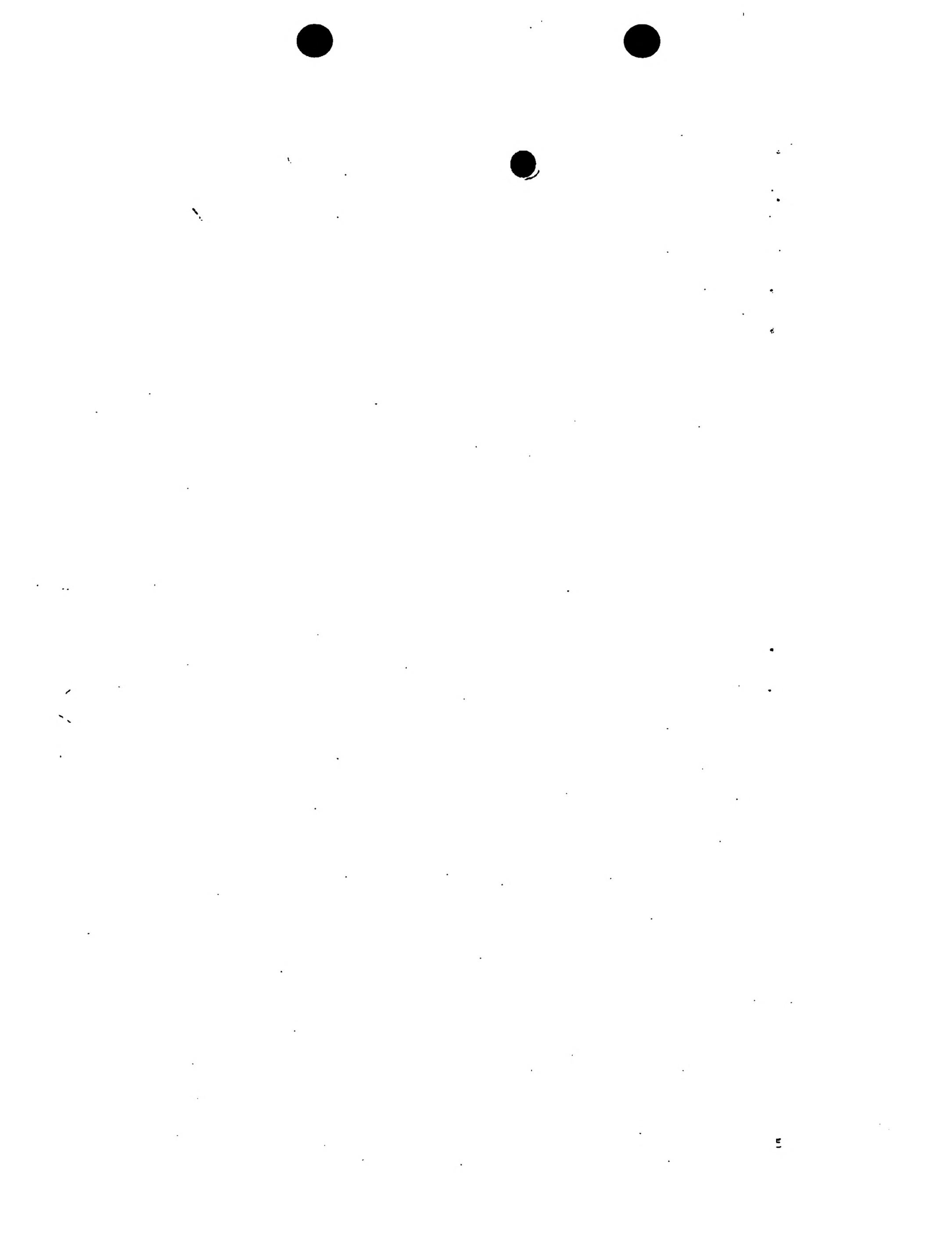


FIG. 4b



WO 93/11755

PCT/US92/11214

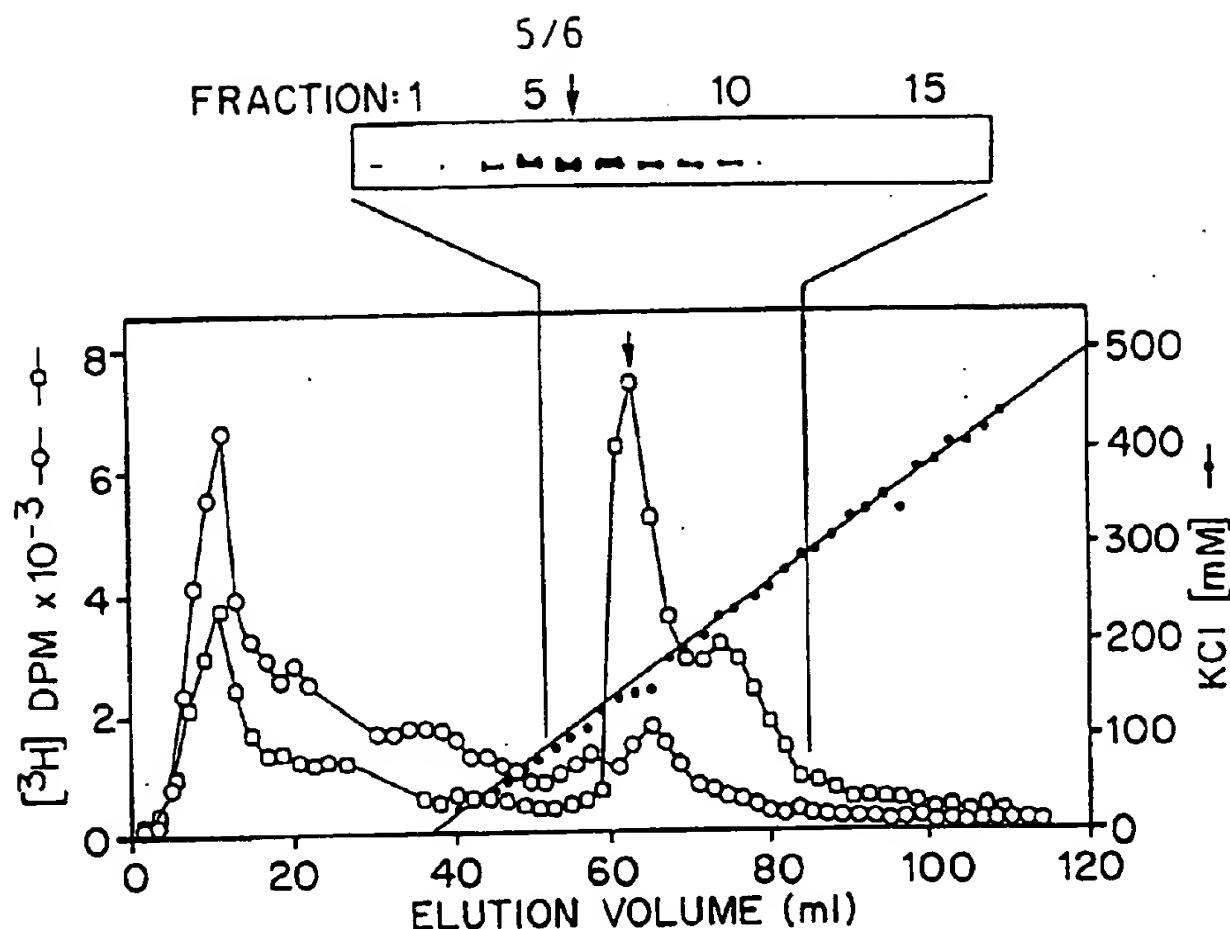


FIG. 5a

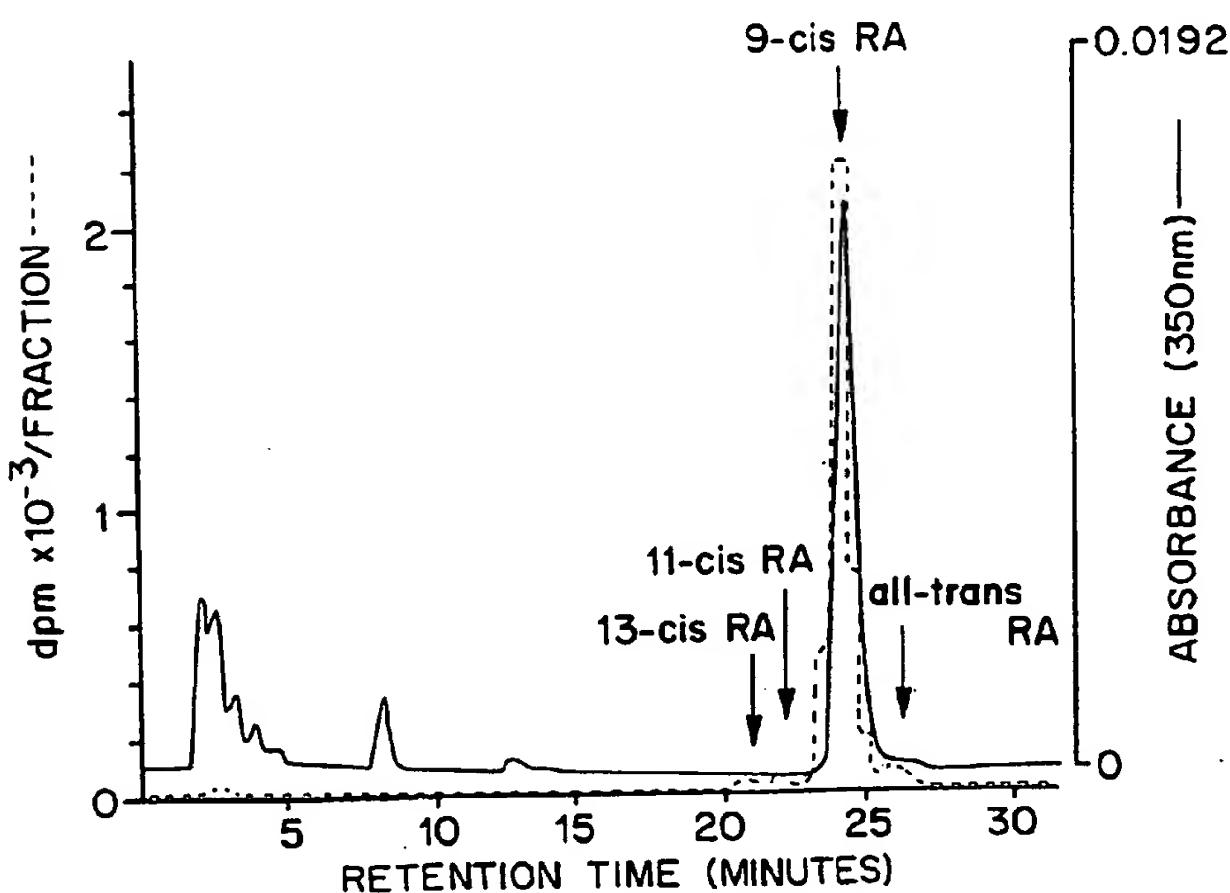
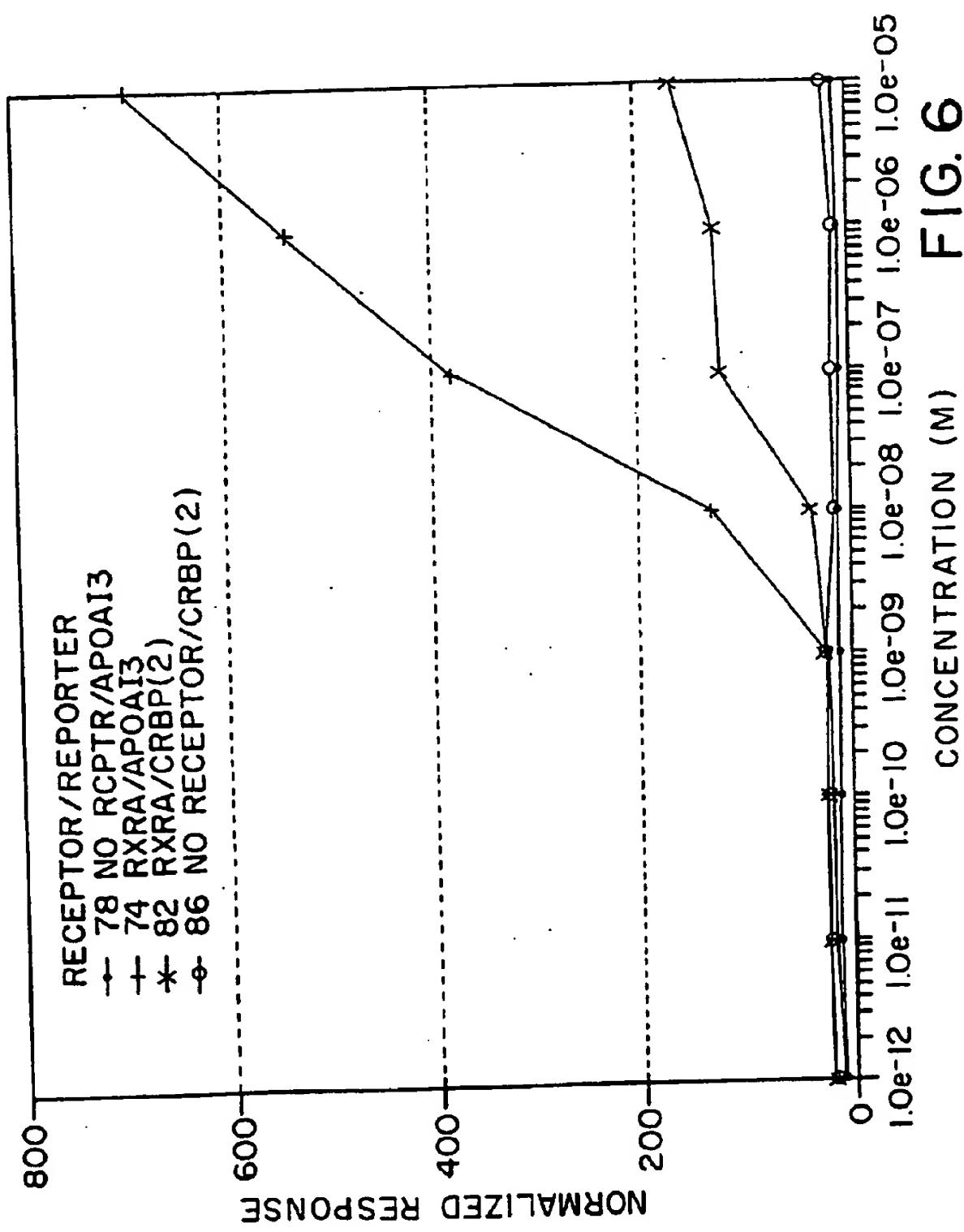


FIG. 5b
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6/6



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/11214

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A61K31/07; C07C403/20		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ; C07C ; C12P ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, if with indication, where appropriate, of the relevant passages ¹¹	Relevant to Claim No. ¹²
X	EP,A,0 376 821 (L'OREAL, FR) 4 July 1990 See the whole document, especially page 7, line 16, the claims ---	28,29
X	FR,A,2 619 309 (L'OREAL, FR) 17 February 1989 See the whole document , especially page 7 line 35, the claims ---	28,29
P,X	JP,A,4 253 934 (NISSHIN FLOUR MILLING CO, LTD) 9 September 1992 see the whole document ---	28-29 -/-
<p>¹⁰ Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed</p> <p>¹¹ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family</p>		
IV. CERTIFICATION		
2	Date of the Actual Completion of the International Search 10 MAY 1993	Date of Mailing of this International Search Report 13.6.93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer S.A. NAUCHE

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	<p>NATURE. vol. 355, 23 January 1992, LONDON GB pages 359 - 361 Levin AA;Sturzenbecker LJ;Kazmer S;Bosakowski T;Huselton C;Allenby G;Speck J;Kratzeisen C;Rosenberger M;Lovey A;et al; '9-cis retinoic acid stereoisomer binds and activates the nuclear receptor RXR alpha.' see the whole document ---</p>	1-22
P,X	<p>CURRENT BIOLOGY vol. 2, no. 6, June 1992, pages 293 - 295 Laudet, V. et al.; 'Nuclear receptors : Flexible friends' see the whole document ---</p>	1-22
P,X	<p>CELL vol. 68, no. 2, 24 January 1992, CAMBRIDGE, MA US pages 397 - 406 Heyman RA;Mangelsdorf DJ;Dyck JA;Stein RB;Eichele G;Evans RM;Thaller C; '9-cis retinoic acid is a high affinity ligand for the retinoid X receptor.' see the whole document ---</p>	1-22
A	<p>THE JOURNAL OF CELL BIOLOGY vol. 99, no. 4, October 1984, NEW YORK, USA page 153A Yen, A. et al.; 'Retinoic acid induced HL-60 Myeloid differentiation sensitivity of early and late events to Cis-Trans isomerisation.' See abstract 563 ---</p>	1-22
A	<p>LEUKEMIA RESEARCH vol. 10, no. 6, 1986, OXFORD, GB pages 619 - 629 YEN, A. ET AL.; 'Retinoic acid induced HL-60 myeloid differentiation : dependence of early and late events on isomeric structure' -----</p>	1-22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/11214

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-3, 6-24 all partially, and claims 5,25 both completely, are directed to a method of treatment of the human/animal body (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 9211214
SA 69151

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 10/05/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0376821	04-07-90	FR-A-	2641184	06-07-90
		CA-A-	2006891	30-06-90
		JP-A-	2221217	04-09-90
		US-A-	5192534	09-03-93
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FR-A-2619309	17-02-89	LU-A-	86969	08-03-89
		AU-B-	626068	23-07-92
		AU-A-	2062288	16-02-89
		BE-A-	1001056	20-06-89
		CH-A-	676422	31-01-91
		DE-A-	3827467	23-02-89
		GB-A,B	2208601	12-04-89
		JP-A-	1156921	20-06-89
		NL-A-	8801963	01-03-89
		SE-A-	8802869	13-02-89
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JP-A-4253934	09-09-92	None		-----
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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 31/07, C07C 403/20		A1	(11) International Publication Number: WO 93/11755 (43) International Publication Date: 24 June 1993 (24.06.93)
(21) International Application Number: PCT/US92/11214 (22) International Filing Date: 18 December 1992 (18.12.92) (30) Priority data: 07/809,980 18 December 1991 (18.12.91) US		(72) Inventors: and (75) Inventors/Applicants (<i>for US only</i>): EVANS, Ronald, M. [US/US]; 8615 La Jolla Scenic Road, La Jolla, CA 92037 (US). MANGELSDORF, David, J. [US/US]; 4771 Seaford Place, San Diego, CA 92117 (US). HEYMAN, Richard, A. [US/US]; 147 Honeycomb Court, Encinitas, CA 92024 (US). BOEHM, Marcus, F. [US/US]; 4007 Everts Street, 4J, San Diego, CA 92109 (US). EICHELE, Gregor [CH/US]; THALLER, Christina [FR/US]; 2030 Swift Boulevard, Houston, TX 77030 (US). (74) Agent: REITER, Stephen, E.; Pretty, Schroeder, Bruegeman & Clark, 444 South Flower Street, Suite 2000, Los Angeles, CA 90071 (US). (81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(71) Applicants (<i>for all designated States except US</i>): THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, La Jolla, CA 92037 (US). BAYLOR COLLEGE OF MEDICINE [US/US]; 1 Baylor Plaza, Houston, TX 77030 (US). LIGAND PHARMACEUTICALS, INC. [US/US]; 9393 Town Centre Drive, Suite 100, San Diego, CA 92121 (US).		Published <i>With international search report.</i> <i>With amended claims.</i> Date of publication of the amended claims: 5 August 1993 (05.08.93)	
(54) Title: MEANS FOR THE MODULATION OF PROCESSES MEDIATED BY RETINOID RECEPTORS AND COMPOUNDS USEFUL THEREFOR			
(57) Abstract <p>In accordance with the present invention, there are provided methods to modulate processes mediated by retinoid receptors, employing high affinity, high specificity ligands for such receptors. In one aspect of the present invention, there are provided ligands which are more selective for the retinoid X receptor than is retinoic acid (i.e., retinoids). In another aspect of the present invention, alternative ligands (other than retinoic acid) have been discovered which are capable of inducing retinoic acid receptor mediated processes. In yet another aspect, methods have been developed for the preparation of such retinoid receptor ligands from readily available compounds.</p>			

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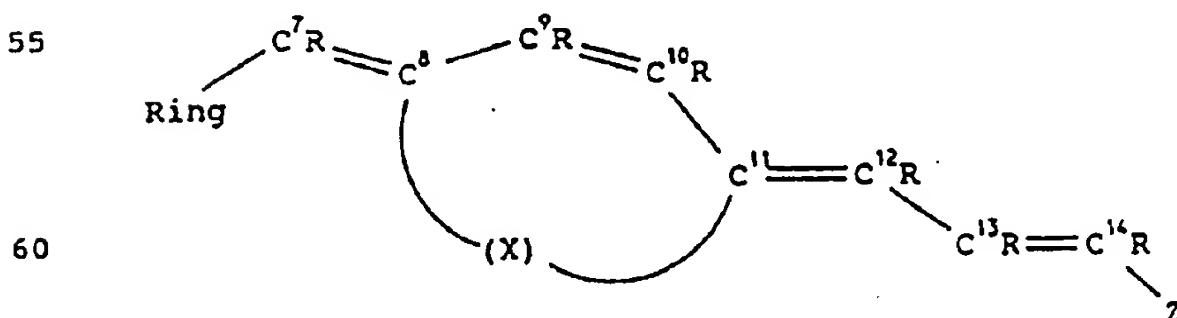
AMENDED CLAIMS

[received by the International Bureau on 13 July 1993 (13.07.93);
original claim 28 amended; remaining claims unchanged (1 page)]

above], thioesters of thioalkyl groups
[-(CR'₂)_n-S-CS-R'], wherein R' and n are as defined
above], aminoalkyl [- (CR'₂)_n-NR'₂, wherein R' and
n are as defined above], N-acyl aminoalkyl
[-(CR'₂)_n-NR'-CO-R"], wherein R' and n are as
defined above and R" is a lower alkyl or benzyl],
carbamate [- (CR'₂)_n-NR'-CO-OR'] or
-(CR'₂)_n-O-CO-NR'₂, wherein R' and n are as
defined above]; and

each R is independently selected from H,
halogen, alkyl, aryl, hydroxy, thiol, alkoxy,
thioalkoxy, amino, or any of the 2 substituents,
with the proviso that Structure A is not
50 9-cis-retinoic acid or 9,13-dicis-retinoic acid;
or

any two or more of the R groups can be
linked to one another to form one or more ring
structures;

Structure I:

wherein:

"Ring", Z and R are as defined above;

65 X is -[(CR₂)_x-X'-(CR₂)_y]-,

X' is selected from -O-, carbonyl, -S-, -S(O)-, -

S(O)₂-, thiocarbonyl, -NR"-, or -CR₂-,

R" is hydrogen, alkyl, hydroxy, thiol, or alkoxy
acyl;

70 x is 0, 1 or 2,

y is 0, 1, or 2, and

x + y ≤ 2;

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